EXERCISE AS A COUNTERMEASURE TO HUMAN AGING

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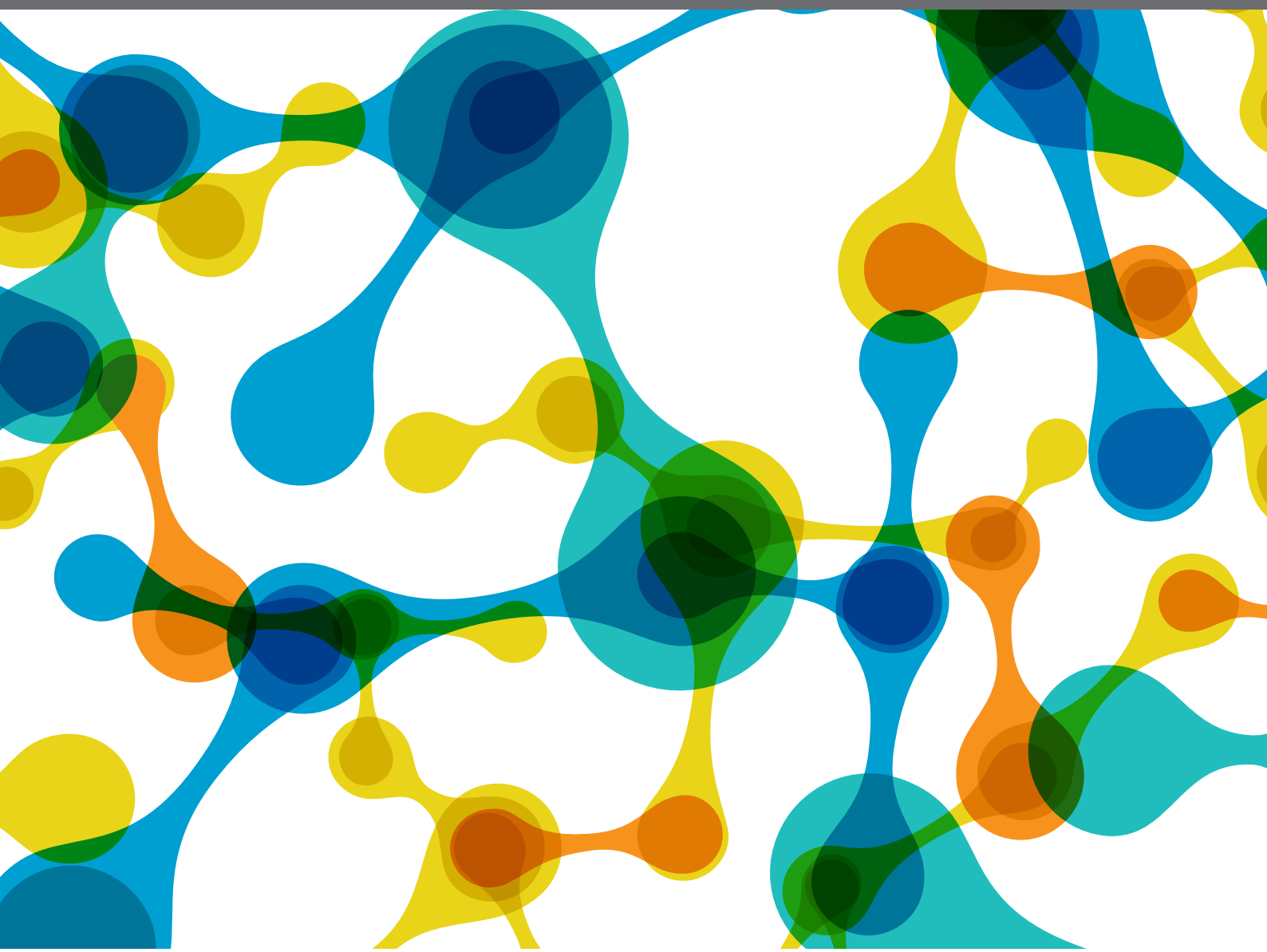

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EXERCISE AS A COUNTERMEASURE TO HUMAN AGING

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EDITORIAL
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Editorial: Exercise as a Countermeasure to Human Aging

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Keywords: exercise, physical activity, aging, lifespan, healthspan

Editorial on the Research Topic

Exercise as a Countermeasure to Human Aging

Unlike many of the branches of natural sciences, there are few true "laws" of physiology. However, there are intrinsic theories about which we are reasonably certain. For example, it is a reasonable statement that exercise and physical activity, in all their forms, typically have positive effects on health and wider physiological function via multiple complex and interacting mechanisms (that we have not yet completely defined). Alternatively, the continuous process of human aging in the adult involves a gradual decline of physiological function across most tissues and systems, again in a complex and intertwining manner. At a point where the average age of humanity is greater than it has ever been, and is continuing to increase, we considered it timely to examine the crossover between these two interacting fields of physiology. Indeed, where past successes in physiology research have emerged from research on transmittable diseases, vaccinations and preventive medicines, our current approaches must now focus on non-transmittable disorders, including frailty syndromes, sarcopenia, and chronic conditions that associate with aging, including heart disease, neuro-cognitive disorders, and diabetes.

When we made this call for submissions, we did not expect the volume of responses we received. In these papers we presented a Research Topic of over 30 articles that covered the interplay between exercise and aging, utilizing approaches that spanned molecular, physiological, and population scale approaches, in both healthy older populations and certain disease subsets, and spanned three *Frontiers* journals *(Frontiers in Physiology, Frontiers in Sports and Active Living, and Frontiers in Aging Neuroscience*). It is a pleasure to note this range of fields and methodological approaches that authors have used.

It has long been known that exercise benefits human function, and that this effect may promote good health into older age. Philostratus (c. 170–250) wrote of individuals who exercised into older age that "They were healthy and did not get sick easily. They stayed youthful into old age, and competed in many Olympics, some in eight and others in nine" (Gymnasticus, p. 44). Several papers in this Research Topic examined classical exercise physiology approaches of a short-term training programme over weeks-to-months. In this vein Kirk et al. gave preliminary results from the LHU-SAT trial, examining 16 weeks of training with or without protein supplementation in healthy over 60-year-old participants. While both groups improved with training, results suggested the protein supplementation group did not improve to a greater degree than the no protein group. However, compliance to protein supplementation beverages in this population continued to be low, an area that may need attention. In line with these results, positive outcomes from classical exercise physiology training interventions were seen by Walker et al. who reported on improved intermuscular coherence, Gavin et al. who noted resistance training improved stair climbing biomechanics in older individuals, Tam et al. reported on resistance training improving exercise economy, and Saeidi et al. findings that a proposed antioxidant altered resistance training-induced

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changes in circulating adipokines in postmenopausal women. Two exercise physiology interventional papers of note include Franchi et al., who used a novel trampoline plyometric training model in a safe and highly effective way, and Jabbour and Majed with the important observation that the widely used ratings of perceived exertion (RPE) scale over-estimated exercise intensity in sedentary older adults. In meta-analyses reviewing exercise changes from short-term training interventions, endurance exercise decreased pro-inflammatory cytokines concentrations (Zheng et al.), yet counterintuitively testosterone was not improved following training studies in older men (Hayes and Elliott), suggesting resistance training-induced benefits were not via circulating testosterone concentrations.

Updating us on recent advances in targeting mitochondria to offset sarcopenia, Coen et al. reviewed exercise and mitochondrial health for successful aging, reminding the reader that exercise is (for now) the only effective option for treatment of sarcopenia. Linking well to this review, Ubaida-Mohien et al. reported on a proteomic analysis of muscle biopsies from 60 individuals spanning 20–87 years of age, and reported physical activity associated with alterations in proteins governing mitochondria energetics, muscle function, gene health, immunity and senescence, and these changes typically opposed those seen with aging. Mirroring these results, ambulatory older individuals presented a preservation in portions of the myostatin and IGF-I signaling pathways, as well as myocyte structures, that wheelchair bound older individuals did not show (Naro et al.). Differing endurance exercise stimuli improved markers of t-cell senescence (Philippe et al.), while in older rats, muscle protein synthesis responses were blunted relative to younger animals (West et al.). All these results point to an environment that is capable of positively responding to anabolic stimuli, but perhaps not as well as younger muscle tissue, as well as a need for research to separate effects of aging and inactivity.

From a population health point of view, increased lifelong activity, not just short-term exercise interventions, are needed. Thus, there has been much recent interest in examining highly trained masters athletes, as a physiological model of successful aging (Pollock et al., 2015; Elliott et al., 2017). This Research Topic included five reports on lifelong exerciser cohorts. Mancini et al. compared lifelong football players with age matched controls, noting a positive influence of lifelong exercise on markers of auto-lysosomal and proteasomal-mediated processes, while Piasecki M. et al. noted an interesting compensatory mechanism whereby power trained older adults showed increased motor unit size, possibly to compensate for decreased motor unit number. In older females, osteoporosis is often seen, however Onambele-Pearson et al. observes that simple mechanical loading is not sufficient to explain bone density, and that fuller measures of activity and inactivity should be considered. In masters athletes who were grouped as "early" or "late" starters to masters athletics (either lifelong training history or beginning after 50 years of age), Piasecki J. et al. reported no major differences in body composition or bone density between these early and late starters, but both groups reliably demonstrated a healthier phenotype vs. inactive controls. Finally, it was of interest to note positive emotional and cognitive effects of lifelong Tai Chi participation relative to an age-matched control group, which was paired with resting-state fMRI connectively differences (Liu et al.). It can be seen that lifelong activity promotes multiple physiological benefits in an aging population.

At one end of the population size spectrum, Knechtle et al. presented a case study on physiological responses in a 95-year-old masters athlete during a 12 h ultra-marathon event. At the other end are population scale studies. It is of interest to note differences in the association between physical activity, as measured by accelerometery, and relative telomere lengths, with positive associations seen in men but not in woman, across a population of 700 older participants (Stenbäck et al.). By analyzing records of ∼27,000 track and field athletes, Ganse et al. observed decreases across maximal power, strength, and endurance records throughout adult lifespan. Further, these declines in performance accelerated post 70 years of age, an observation that was seen in grip strength in the general population (Dodds et al., 2014), and occurred despite high levels of physical activity. These results, in combination, suggested that muscle function loss with age is not only inactivity-induced but has an intrinsic component.

As aging is associated with an increased risk of cardiovascular disease, diabetes and certain types of cancers, and chronic exercise associates with reduced rates of such disorders, it is important to examine exercise in such older populations with such conditions. Indeed, regular exercise training of any type improved quality of life, aerobic capacity and heart function in older heart failure patients (Slimani et al.). Mcleod et al. argued for alterations in guidelines for exercise in the prevention of chronic disorders, promoting the role of resistance training in preventive medicine, interesting reading when paired with the Campbell et al. meta-analysis which observed insufficient evidence to recommend aerobic exercise for vascular function improvement in older sedentary adults. In rats, experimental data suggested that prior exercise training improved survivability from experimental coronary artery occlusion (Veiga et al.), providing us humans with more motivation for maintaining lifelong exercise. This was reinforced by a cohort study of ∼3,700 individuals, where both physical activity and sedentary time both independently predicted mortality rates associated with pro-inflammatory conditions (Cabanas-Sánchez et al.). Other findings suggested the improvements in post-exercise reaction time were not different between hypertensive and non-hypertensive patients (Lefferts et al.), and the interesting observation that structural differences in skeletal muscle may underlie difference in stretch shorten cycle between COPD patients and healthy age-matched controls (Navarro-Cruz et al.). These results reinforce the recent American Medical Association's guidelines promoting exercise wherever possible in chronic conditions (Piercy et al., 2018).

Historically, physiology research has primarily utilized the "healthy young male" population, thus we are pleased to note that 14 of the 21 primary experimental papers presented here in human participants included male and female groups, while one specifically examined post-menopausal changes in women. Likewise, we feel the papers presented here give valuable insight

concerning the range of aging physiology, in a continuous rather than dichotomous manner. For example, Knechtle et al. concerned a 95-year-old masters athlete, considered the 'oldest old', whereas some papers (Hayes and Elliott) had a minimum age of 60, considered the 'young old'. Moreover, several investigations utilized a young comparison group or a cross sectional design, which permitted authors to study life course aging utilizing multiple research designs.

Both physical activity and structured exercise are near-uniformly positive for human longevity and well-being by multiple, complex physiological mechanisms and pathways that help maintain health, independence and quality of life. Indeed, the complexity of the aging process and the role of exercise in aging physiology were well-represented by the diversity of experimental approaches witnessed in this Research Topic. Combined, the results of these investigations suggested that exercise and activity can offset decreases in human function that we consider "inevitable aspects of aging" but cannot prevent them completely. Our understanding of how and why exercise and activity promote healthy aging, and indeed the basic physiology of the aging process, is currently incomplete. It is our aim that this Research Topic makes a small contribution to the understanding of this complex field.

AUTHOR CONTRIBUTIONS

BE wrote the first draft. LH, DH, and MB critically reviewed, and all authors approved the final version of this editorial.

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ORIGINAL RESEARCH
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Physical Activity, Sitting Time, and Mortality From Inflammatory Diseases in Older Adults

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Objective: The aim of this study was to examine the independent and combined associations of physical activity (PA) and sitting time (ST) with long-term mortality attributed to inflammatory causes other than cardiovascular disease (CVD) and cancer in a national cohort of older adults in Spain.

Design: Prospective study.

Setting and Participants: A cohort of 3,677 individuals (1,626 men) aged ≥60 years was followed-up during 14.3 years.

Measures: At baseline, individuals reported PA and ST. The study outcome was death from inflammatory diseases when CVD or cancer mortality was excluded. This outcome was classified into infectious and non-infectious conditions. Analyses were performed with Cox regression and adjusted for PA, ST, and other main confounders (age, sex, educational level, smoking, alcohol consumption, body mass index, and chronic conditions).

Results: During follow-up, 286 deaths from inflammatory diseases (77 from infectious diseases) were identified. Compared to individuals who defined themselves as inactive/less active, mortality from inflammatory diseases was lower in those who were moderately active (hazard ratio [HR] = 0.67, 95% confidence interval [CI] = 0.50–0.90) or very active (HR = 0.48, 95%CI = 0.33–0.68), independently of ST. Also, being seated ≥7 h/d vs. <7 h/d was linked to higher mortality (HR = 1.38, 95%CI = 1.02–1.87). The largest risk of mortality was observed in inactive/less active individuals with ST≥7 h/d (HR = 2.29, 95%CI = 1.59–3.29) compared to those with moderate/very PA and ST <7 h/d. Low PA and high ST were consistently associated with a higher risk of mortality from non-infectious inflammatory causes. Associations of PA and ST with mortality from infectious inflammatory causes showed a similar trend, but most of them did not reach statistical significance.

Conclusions: Low PA and high ST were independently associated with higher mortality from inflammatory diseases other than CVD or cancer in older adults. Interventions addressing simultaneously both behaviors could have greater benefits than those focusing on only one of them.

Keywords: physical activity, sitting time, mortality, inflammation diseases, infectious diseases

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INTRODUCTION

Inflammation is a physiological response to tissue injuries or infections (Addison et al., 2012). However, when inflammation persists, chronically elevated systemic levels of pro-inflammatory cytokines can lead an increased risk of inflammatory diseases. In this sense, chronic inflammation is known to play a central role in the etiology of cardiovascular disease (CVD) and cancer (Singh-Manoux et al., 2017) and plays a major pathogenic role in other diseases such as diabetes, chronic kidney disease, inflammatory bowel, cirrhosis, chronic obstructive pulmonary disease, rheumatoid arthritis, and several neurodegenerative conditions (Dungey et al., 2013; Holmes, 2013; Wang et al., 2013).

Aging is a progressive process associated with several physiological changes, chronic inflammation and a deterioration in homeostatic functions, which contributes to increased oxidative stress (Salminen et al., 2012). Indeed, compared with young or middle-age adults, older populations have an increased incidence of infectious and other inflammatory conditions (Bender, 2003).

There is evidence that regular physical activity (PA) produces anti-inflammatory actions that might prevent cardiovascular disease (CVD), cancer and many other age-associated conditions, including mitochondrial dysfunction, frailty, sarcopenia, and physical and mental disability (Chen et al., 2014; Sallam and Laher, 2016). By contrast, sedentary behavior, defined as any waking behavior characterized by low levels of energy expenditure, is linked to low-grade inflammation, independently of PA or adiposity (Henson et al., 2013). Although the effect of PA and sitting time (ST) on CVD and cancer mortality has been widely studied in adults (Nocon et al., 2008; Katzmarzyk et al., 2009; Wilmot et al., 2012), no previous investigation has focused on mortality from other diseases with a major inflammatory component in older adults. Accordingly, this work examined the independent and combined association of PA and ST with long-term mortality from inflammatory diseases, after excluding CVD or cancer mortality, in a national cohort of older adults.

METHODS

Study Design and Participants

We analyzed data from the *Universidad Autónoma de Madrid* (UAM) cohort with a total of 4,008 individuals (1,739 men) representative of the non-institutionalized population aged 60 years and older in Spain. The study methods have been reported elsewhere (León-Muñoz et al., 2013; Martínez-Gómez et al., 2015). In short, participants were recruited between October 2000 and February 2001 using probabilistic sampling by multistage clusters. The clusters were stratified according to region of residence and size of municipality. Then, census sections and households were chosen randomly within each cluster. Finally, study participants were selected from 420 census sections in sex and age (60–69, 70–79, and $\geq$80 years) strata. The information was collected through home-based personal interviews using a structured questionnaire, followed by a physical examination performed by trained and certified personnel. The study response rate was 71%. The study was approved by the Clinical Research Ethics Committee of *La Paz* University Hospital (Madrid, Spain), and written informed consent was obtained from all study participants and an accompanying family member.

Physical Activity and Sitting Time

Information on PA and ST was self-reported. PA was obtained with a global question that asked participants to rate their level of PA in comparison with their age-peers in four categories: inactive, less active, moderately active, and very active (Martínez-Gómez et al., 2015). Because the prevalence of the lowest PA category was 7.21%, those belonging to the categories "inactive" and "less active" were merged into the same one.

ST was estimated by leisure time spent sitting down based on the following question (León-Muñoz et al., 2013): *"About how much time per day do you spend sitting down on weekdays? Please add up the total number of hours that you spent sitting down regardless of the activity that you do (eating, listening to the radio, watching television, reading, sewing, driving, etc.)*." The same question was asked with reference to weekend days. The number of hours per day was calculated as follows: [(weekday STx5 + weekend day STx2)/7]. ST was classified into tertiles with cut-points at 3.29 and 5.29 h/d.

Mortality

All-cause deaths among study participants, from study baseline at 2000/2001 to the end of follow-up at 31 December 2015, were identified by a computerized search of the National Death Index, which contains information on the vital status of all residents in Spain. The vital status was ascertained for 99.9% of the cohort. We considered inflammatory diseases other than CVD or cancer as those where inflammation or infection play a major pathogenic role; these diseases can be further classified as infectious and non-infectious. In accordance with previous studies (Andersen et al., 2006), we selected deaths with the following codes of the tenth revision of the International Classification of Diseases (ICD-10): (a) Infectious diseases (A04.7, A41.9, B18.2, J18, J18.0, J18.1, J18.9, J22, and N39.0) and (b) non-infectious diseases: chronic neurodegenerative diseases (G20, G30.1, and G30.9), diabetes (E11.9, E14.5, E14.7, and E14.9), chronic lower respiratory diseases (J40, J42, J43.9, J44, J44.1, J44.8, J44.9, J45.9, and J47), cirrhosis (K74.6), kidney failure (N12, N18.9, and N19), other diseases of the respiratory system (J69, J69.0, and J84.1), cholecystitis (K81.9), and other diseases (E85.4 and N32.1).

Covariates

Age and sex were recorded. Educational level was evaluated as the highest level achieved (no formal education, primary, and secondary or higher). Participants also reported whether they were never, former, or current smokers. Alcohol consumption was obtained with the frequency-quantity scale used in the Spanish National Health Survey (Guallar-Castillón et al., 2001). Firstly, individuals rated their alcoholic beverage consumption among the following options: abstainer, former drinker, and current/sporadic drinker. Then, those who indicated current/sporadic drinking also reported the frequency and quantity of beer, wine, and spirits consumed during the past year.

Total alcohol intake was classified into excessive and moderate consumption using cutoff points of >30 g/d in men and >20 g/d in women (Chalasani et al., 2012). Weight and height were measured using standardized procedures (Gutiérrez-Fisac et al., 2004), and the body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared. Finally, information on the following chronic conditions diagnosed by a physician and reported by the study participants was recorded: chronic lung disease, cardiovascular disease, diabetes mellitus, Parkinson, and cancer at any site.

Statistical Analyses

Of the 4,008 participants, 331 were excluded because of missing data on one or more of the study variables. Thus, the analyses were conducted with 3,677 individuals (1,626 men). We initially compared risk estimates for sex and age (<70 and ≥70 years) strata and no significant interactions were observed (all $p > 0.05$); therefore, the analyses were performed for the total sample. Baseline characteristics of the study sample by categories of PA and ST were presented as mean ± SD or %. Cramer's V was used to describe the relationship between groups of PA and ST.

The association of PA (inactive/less active, moderately active, and very active) and ST (tertiles) with mortality from inflammatory diseases was summarized with hazard ratios (HR) and their 95% confidence intervals (CI) obtained from Cox regression. Three models with progressive adjustment for potential confounders were fitted. The first model adjusted for age and sex; the second model further adjusted for the rest of potential covariates; and the third model also included PA or ST (as appropriate) to examine the independent association of both behaviors. The P for trend was calculated by modeling the categories of PA and the tertiles of ST as continuous variables. Moreover, to assess the dose-response relationship between inflammatory mortality and ST as a continuous variable, we fitted a restricted cubic spline, with adjustment as in model 3, testing the departure from a linear trend.

To examine the combined effect of PA and ST on inflammatory mortality, first, we considered as health risk behaviors (HRBs) the categories of PA (inactive/less active) and ST (≥7 h/d) showing a detrimental role on inflammatory mortality in the aforementioned individual associations. Then, we created four categories of health risk behaviors: (1) 0 HRBs: high PA and low ST; (2) 1 HRB (high ST): high PA and high ST; (3) 1 HRB (low PA): low PA and low ST; and (4) 2 HRBs: low PA and high ST. Analyses using those categories were performed with Cox regression and adjusted as in models 1 and 2.

To rule out the effect of subclinical disease on the study results and to reduce the likelihood of reverse causation, we replicated the analyses after excluding 200 individuals who died during the two first years of follow-up.

We assessed the assumption of the proportionality of mortality hazards both graphically and by testing the significance of interaction terms for the main exposure variables and years of follow-up. No evidence was found of departure from the proportional hazards assumption ($P > 0.05$).

Analyses were performed with STATA® v.14.0, and statistical significance was set at $P < 0.05$.

RESULTS

The main characteristics of the study participants at baseline are shown in **Table 1**. Differences in all variables were identified among the PA level groups (all $P < 0.01$). Compared to those in the lowest tertile of ST, those in the highest tertile were older, showed lower educational level, were more likely to be never drinkers, had higher BMI, and had suffered more frequently from coronary heart disease or diabetes (all $P < 0.05$). PA and ST were weakly associated (*Cramer's* $V = 0.245$, $P < 0.05$). Interaction between PA and ST was close to significance (i.e., $P = 0.063$ for inflammatory causes mortality).

During a mean follow-up of 10.77 years (median = 13.73 years, range, 0.02–14.25), corresponding to 50,485 person-years, 1,669 (45.39%) deaths occurred. Among them, there were 286 inflammatory deaths other than CVD or cancer: 77 were from infectious diseases and 209 from non-infectious diseases (Supplementary Table 1).

A higher PA level was associated with a progressively lower risk of death from total inflammatory diseases ($P < 0.001$) (**Table 2**). In fully adjusted models, compared to inactive/less active individuals, the HR (95% CI) of mortality from inflammatory diseases was 0.67 (0.50–0.90) in those who were moderately active and 0.48 (0.33–0.68) in those who were very active. Results were similar for infectious and non-infectious diseases, but they achieved statistical significance only for non-infectious diseases.

ST showed a direct dose-response relationship with mortality from inflammatory diseases, although it lost statistical significance after adjustment for PA (**Table 2**). In spline analyses (**Figure 1**), the association between ST and mortality showed a non-lineal trend (all $P < 0.01$), with mortality risks associated with ≥7 h/d in ST. This non-lineal trend was more evident for mortality attributed to infectious inflammatory causes. In fully adjusted models, compared to those with ST <7 h/d, participants with ST ≥7 h/d had a mortality HR (95%CI) of 1.38 (1.02–1.87) and 1.46 (1.02–2.10) for total and non-infectious inflammatory causes, respectively; the corresponding result for infectious diseases was 1.15 (0.68–1.97).

Table 3 shows the risk of mortality according to the number and type of HRBs. A HRB was defined as low PA (being inactive/less active) or high ST (≥7 h/d). The percentage of individuals with 0 HRBs, 1 HRB (high ST), 1 HRB (low PA), and 2 HRBs (high ST and low PA) was respectively 66.5, 8.4, 14.6, and 10.4%. In fully adjusted analyses, and compared with participants with 0 HRBs, the HR (95%CI) for mortality from inflammatory diseases was 1.45 (0.97–2.18) for individuals with 1 HRB (high ST), 1.68 (1.19–2.37) for individuals with 1 HRB (low PA), and 2.29 (1.59–3.29) for individuals with 2 HRBs (high ST and low PA). Results were very similar for non-infectious disease mortality. As regard to mortality from infectious diseases, results were mostly in the same direction although they were fairly imprecise, and did not achieve statistical significance.

Results remained virtually identical after excluding deaths in the first two years of follow-up ($n = 31$ for all non-CVD/cancer inflammatory causes); for example, the adjusted HRs (95%CI)

TABLE 1 | Characteristics of study participants at baseline.

	All	Physical activity				Sitting time			
		Inactive/less active	Moderately active	Very active	*P*	Tertile 1 (lowest)	Tertile 2	Tertile 3 (highest)	*P*
N	3,677	920	1,576	1,181	–	1,297	1,181	1,199	–
Age (years), mean ± *SD*	71.72 ± 7.90	74.77 ± 8.26	71.10 ± 7.66	70.17 ± 7.28	< 0.001	70.01 ± 7.34	71.13 ± 7.47	74.15 ± 8.31	< 0.001
Men, %	44.22	37.15	43.98	50.04	< 0.001	44.55	45.31	42.79	0.347
EDUCATIONAL LEVEL, %									
No education	51.84	61.55	51.56	44.65	< 0.001	48.29	49.11	58.35	< 0.001
Primary	34.79	28.83	34.85	39.34		38.88	36.24	28.93	
Secondary or higher	13.38	9.61	13.60	16.01		12.83	14.65	12.72	
SMOKING STATUS, %									
Never	65.34	67.99	66.26	62.07	0.001	66.25	64.93	64.78	0.627
Former	24.33	23.90	23.96	25.15		24.11	23.47	25.40	
Current	10.33	8.10	9.79	12.79		9.64	11.60	9.83	
ALCOHOL CONSUMPTION, %									
Never	49.32	55.83	51.12	41.84	< 0.001	42.77	51.14	54.61	< 0.001
Former	11.92	16.21	11.10	9.67		9.96	11.06	14.88	
Moderate[I]	28.59	21.73	27.39	35.52		35.87	27.22	22.06	
Heavy[I]	10.18	6.22	10.39	12.97		11.40	10.59	8.45	
Body mass index (kg/m^2), mean ± *SD*	28.84 ± 4.60	29.43 ± 5.39	28.90 ± 4.42	28.30 ± 4.07	< 0.001	28.71 ± 4.34	28.73 ± 4.34	29.10 ± 5.09	0.034
Chronic lung disease, %	14.09	21.05	12.81	10.39	< 0.001	12.07	12.15	18.20	0.089
Coronary heart disease, %	8.61	14.50	7.47	5.54	< 0.001	5.79	8.87	11.40	< 0.001
Diabetes mellitus, %	15.29	20.02	15.40	11.46	< 0.001	12.70	15.32	18.06	0.011
Parkinson, %	1.46	2.87	0.92	1.08	< 0.001	1.10	0.90	2.40	0.075
Cancer, %	1.79	3.11	1.11	1.66	0.006	1.60	1.37	2.40	0.908
PHYSICAL ACTIVITY, %									
Inactive/low active	25.01	–	–	–	–	14.66	15.81	45.26	< 0.001
Moderately active	42.87	–	–	–		42.37	49.53	36.84	
Very active	32.12	–	–	–		42.97	34.66	17.89	
Sitting time (h/d), mean ± *SD*	4.79 ± 2.74	6.48 ± 3.61	4.47 ± 2.08	3.91 ± 2.09	< 0.001	2.39 ± 0.71	4.38 ± 0.52	7.80 ± 2.59	< 0.001

[I] *Threshold between moderate and heavy drinker: 10g*d^{-1} in women and 20 g*d^{-1} in men.*

of mortality for all inflammatory causes in those with 1 HRB (low PA) and 2 HRBs were respectively 1.66 (1.16–2.37) and 2.08 (1.39–3.12).

DISCUSSION

In this prospective study with older adults from Spain we found dose-response associations of PA and ST with mortality from inflammatory causes other than CVD and cancer. Specifically, participants who were moderately active or very active had lower mortality risk compared to individuals who were inactive/less active, regardless of the amount of ST. Additionally, mortality risk was higher among participants who reported high ST (≥7 h/d) compared with participants having lower levels of ST, independently of PA. Results were in the same direction for infectious and non-infectious diseases, though associations were stronger and achieved statistical significance only for non-infectious diseases.

A proinflammatory state, also called "*inflamm-aging*," is associated with aging and may contribute to many disabling diseases (Sallam and Laher, 2016). However, although inflammatory cytokines increase with age, chronic inflammation might not be necessarily a manifestation of aging "*per se*" and some lifestyle factors, such as PA or ST, may play a prominent role. In this sense, in older adults, regular PA has been systematically linked to reductions in inflammatory biomarkers, including IL-6, TNF-α, and C-reactive protein

TABLE 2 | Mortality risk for inflammatory causes according to levels of physical activity and sitting time in older adults.

	Physical activity (categories)				Sitting time (tertiles)			
	Inactive/less active	Moderately active	Very active	P for trend	Tertile 1 (lowest)	Tertile 2	Tertile 3 (highest)	P for trend
N	920	1,576	1,181		1,297	1,181	1,199	
INFLAMMATORY CAUSES (*n* = 286)								
Deaths	110	117	59		77	82	127	
Model 1, HR (95% CI)	1.00 (Ref.)	0.61 (0.46–0.82)	0.41 (0.29–0.58)	<0.001	1.00 (Ref.)	1.12 (0.79–1.57)	1.61 (1.17–2.21)	0.003
Model 2, HR (95% CI)	1.00 (Ref.)	0.64 (0.47–0.86)	0.44 (0.31–0.63)	<0.001	1.00 (Ref.)	1.07 (0.75–1.53)	1.49 (1.08–2.06)	0.014
Model 3, HR (95% CI)	1.00 (Ref.)	0.67 (0.50–0.90)	0.48 (0.33–0.68)	<0.001	1.00 (Ref.)	1.06 (0.74–1.51)	1.28 (0.92–1.77)	0.140
INFECTIOUS INFLAMMATORY CAUSES (*n* = 77)								
Deaths	27	33	17		22	25	30	
Model 1, HR (95% CI)	1.00 (Ref.)	0.81 (0.48–1.37)	0.56 (0.30–1.04)	0.063	1.00 (Ref.)	1.19 (0.64–2.20)	1.22 (0.68–2.17)	0.507
Model 2, HR (95% CI)	1.00 (Ref.)	0.83 (0.49–1.40)	0.58 (0.31–1.09)	0.088	1.00 (Ref.)	1.20 (0.64–2.27)	1.22 (0.68–2.19)	0.509
Model 3, HR (95% CI)	1.00 (Ref.)	0.83 (0.49–1.41)	0.59 (0.31–1.13)	0.105	1.00 (Ref.)	1.20 (0.64–2.25)	1.12 (0.62–2.05)	0.712
NON-INFECTIOUS INFLAMMATORY CAUSES (*n* = 209)								
Deaths	83	84	42		55	57	97	
Model 1, HR (95% CI)	1.00 (Ref.)	0.55 (0.39–0.79)	0.36 (0.24–0.55)	<0.001	1.00 (Ref.)	1.09 (0.72–1.65)	1.78 (1.23–2.58)	0.002
Model 2, HR (95% CI)	1.00 (Ref.)	0.59 (0.41–0.84)	0.41 (0.27–0.62)	<0.001	1.00 (Ref.)	1.03 (0.67–1.58)	1.59 (1.08–2.33)	0.015
Model 3, HR (95% CI)	1.00 (Ref.)	0.63 (0.44–0.90)	0.45 (0.29–0.69)	<0.001	1.00 (Ref.)	1.01 (0.66–1.55)	1.33 (0.90–1.97)	0.139

HR, Hazard ratio; CI, Confidence Interval. Cut points for sitting time tertiles were 3.29 and 5.29. Model 1 was adjusted for age (years) and sex (male/female). Model 2 was adjusted for age (years), sex (male/female), educational level (no formal studies/Primary studies/Secondary or higher studies), smoking (never smoker/ former smoker/current smoker), alcohol consumption (never drinker/former drinker/moderate drinker/heavy drinker), body mass index (kg/m^2), chronic lung disease (no/yes), cardiovascular disease (no/yes), diabetes mellitus (no/yes), Parkinson (no/yes), and cancer (no/yes). Model 3 was adjusted as in Model 2 plus physical activity or sitting time (as appropriate).

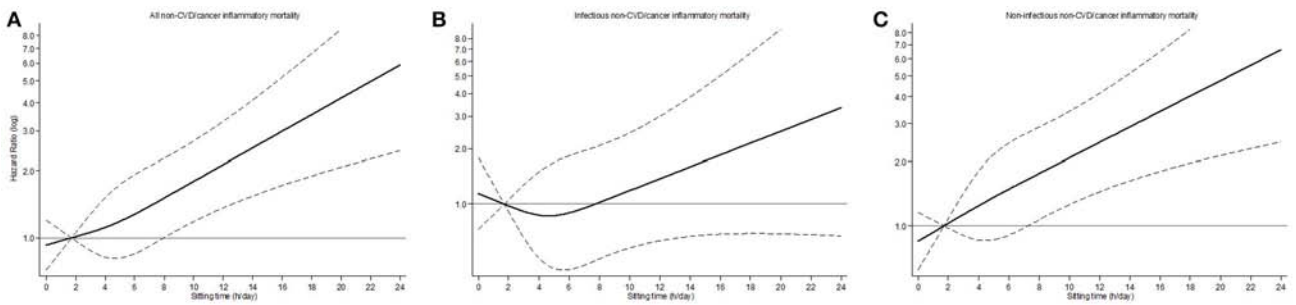

FIGURE 1 | Hazard ratio and 95% confidence interval for mortality for inflammatory causes according to sitting time in older adults: **(A)** All-inflammatory causes. **(B)** Infectious inflammatory causes. **(C)** Non-infectious inflammatory causes. Footnotes: estimates are from Cox regression models of inflammatory, infectious inflammatory and non-infectious inflammatory causes mortality against a restricted cubic spline of sitting time with knots at 2, 4.14, and 8. The black line plots the hazard ratio, and the dashed lines indicate the upper and lower 95% confidence limits. Analyses were adjusted for age (years), sex (male/female), educational level (no formal studies/Primary studies/Secondary or higher studies), smoking (never smoker/ former smoker/current smoker), alcohol consumption (never drinker/former drinker/moderate drinker/heavy drinker), body mass index (kg/m^2), chronic lung disease (no/yes), cardiovascular disease (no/yes), diabetes mellitus (no/yes), Parkinson (no/yes), cancer (no/yes), and physical activity (inactive or less active/moderately active/very active).

(CRP) (Elosua et al., 2005), as well as to increased levels of anti-inflammatory cytokines, such as IL-10 (Jankord and Jemiolo, 2004). Prolonged sedentary behavior has also been associated with higher pro-inflammatory markers, independently of PA (Healy et al., 2011; Gennuso et al., 2013; Henson et al., 2013). Due to the effect of PA and ST on chronic inflammation in older

TABLE 3 | Mortality risk for inflammatory causes according to groups of Health Risk Behaviors (HRBs).

	Health risk behaviors			
	0 HRB (high PA and low ST)	**1 HRB (high PA and high ST)**	**1 HRB (low PA and low ST)**	**2 HRB (low PA and high ST)**
N	2,447	310	538	382
INFLAMMATORY CAUSES (*n* = 286)				
Deaths	145	31	61	49
Model 1, HR (95% CI)	1.00 (Ref.)	1.50 (1.00–2.27)	1.78 (1.27–2.49)	2.46 (1.70–3.54)
Model 2, HR (95% CI)	1.00 (Ref.)	1.45 (0.97–2.18)	1.68 (1.19–2.37)	2.29 (1.59–3.29)
INFECTIOUS INFLAMMATORY CAUSES (*n* = 77)				
Deaths	43	7	15	12
Model 1, HR (95% CI)	1.00 (Ref.)	0.92 (0.40–2.12)	1.22 (0.65–2.28)	1.68 (0.88–3.19)
Model 2, HR (95% CI)	1.00 (Ref.)	0.93 (0.40–2.17)	1.17 (0.62–2.22)	1.67 (0.89–3.15)
NON-INFECTIOUS INFLAMMATORY CAUSES (*n* = 209)				
Deaths	102	24	46	37
Model 1, HR (95% CI)	1.00 (Ref.)	1.78 (1.11–2.85)	2.05 (1.38–3.04)	2.84 (1.83–4.39)
Model 2, HR (95% CI)	1.00 (Ref.)	1.65 (1.03–2.65)	1.88 (1.25–2.83)	2.55 (1.66–3.93)

HR, Hazard ratio; CI, Confidence Interval; ST, Sitting Time; PA, Physical Activity; HRB, Health Risk Behavior. High ST was considered 7 h or more of sitting time. Low PA was considered as inactive or less active. Model 1 was adjusted for age (years) and sex (male/female). Model 2 was adjusted for age (years), sex (male/female), educational level (no formal studies/Primary studies/Secondary or higher studies), smoking (never smoker/ former smoker/current smoker), alcohol consumption (never drinker/former drinker/moderate drinker/heavy drinker), body mass index (kg/m^2), chronic lung disease (no/yes), cardiovascular disease (no/yes), diabetes mellitus (no/yes), Parkinson (no/yes), and cancer (no/yes).

people, it is important to assess the extent of the association between these behaviors and mortality from diseases where inflammation plays an important pathogenic role.

The death rates from inflammation-related diseases others than CVD and cancer are substantial; for example, chronic lower respiratory diseases, Alzheimer's disease, diabetes, and kidney disease are among the 10 leading causes of death in the United States (Xu et al., 2016). Similarly, data from the National Institute of Statistics in Spain (www.ine.es) showed that around 20% of deaths in 2015 among adults over 60 years were due to diabetes, Alzheimer's disease or chronic lower respiratory diseases. This highlights the relevance of exploring the modifiable factors associated with these relevant cause-specific mortalities. To the best of our knowledge, this is the first study to examine the independent and combined association of PA and ST with mortality attributed to all inflammatory causes other than CVD or cancer.

Our results are consistent with those of previous studies on the effect of PA on mortality from some inflammation-related diseases. In the Danish Diet, Cancer and Health cohort, participating in sports, cycling, and gardening was linked to reduced risk of death from diabetes (ranged 39–66%) and respiratory diseases in older adults (ranged 37–40%) (Andersen et al., 2015). Moreover, Rosness et al. (2014) reported that, compared with inactive older individuals, the risk of dementia-related mortality was lower in those who engaged in light or vigorous PA during three or more hours per week. Regarding sedentary behavior, Cucino and Sonnenberg (2001) found that mortality related with inflammatory bowel disease was higher in individuals with sedentary vs. manual occupations. Evidence is slightly greater for TV viewing, the most prevalent sedentary behavior in older people (Keadle et al., 2015; Ukawa et al., 2015). Keadle et al. (2015) identified that each 2 h/d increase in TV viewing was associated with an elevated risk of mortality for chronic obstructive pulmonary disease, diabetes, influenza/pneumonia, Parkinson's, and liver disease. In our study, we found that the association between ST and mortality from inflammatory diseases was not linear and the risk of mortality increased when spending $\geq$7 h/d. Non-linear relationships have also been observed in previous studies examining all-cause deaths; Lee (2016) described that all-cause mortality risk increased linearly when daily sedentary behaviors exceeded 9 h/d. A meta-analysis by Chau et al. (2013) found that the best fitted spline model to characterize the dose-response relationship between sedentary behavior and all-cause mortality was with knots at >3 and >7 h/d.

We also attempted to evaluate the combined effect of PA and ST on inflammatory mortality. When compared to participants who had 0 HRB (high PA and low ST), the mortality risk for inflammatory causes, especially non-infectious causes, was higher in those who presented 1 HRB (i.e., low PA and low ST), and was further increased in individuals with 2 HRB (low PA and high ST). These are in line with earlier research showing the greater protection against morbidity when adequate levels of several lifestyle factors are combined (Sotos-Prieto et al., 2016; Edwards and Loprinzi, 2018). Specifically, our results suggest that interventions addressing concurrently an increase in PA and decrease in ST in older people might have a greater effect on inflammatory survival than those only targeting one of them.

Finally, it is relevant to note that PA and ST were weakly related with mortality attributed to infectious inflammatory causes, so that most associations did not reach statistical significance. The absence of associations may be due to lack of statistical power as the number of deaths related with infectious

inflammatory diseases was low ($n = 77$, 26.9% of the total). On the other hand, it is possible that the protective effect of high PA and low ST on inflammation is insufficient to combat external pathogens, particularly in the elderly because of their reduced immune function and greater vulnerability to multiple infections (Bartlett et al., 2016). In addition, the effects of PA on the immune system could vary with the type, intensity and context of PA (Grosset-Janin et al., 2012). A retrospective study with older participants found no relation between overall PA and the number of upper respiratory tract infections (URTI) episodes, but sports participation was negatively correlated with number of URTI episodes during a 1-year period (Kostka et al., 2000). Unfortunately, information on the type and intensity of PA was not available in our study and future research should address this issue.

Our study has some limitations. First, PA and ST were self-reported, and recall biases might have occurred. No data are available on the reliability and validity of the questions utilized in this study, but similar self-report measures have demonstrated adequate validity and reliability in older adults (Van Cauwenberg et al., 2014). Furthermore, information about diet was not collected, and data on PA and ST was ascertained only at baseline so changes in the levels of these behaviors during the follow-up period may have affected the associations we examined. Lastly, although similar classifications of inflammatory diseases have been used previously (Andersen et al., 2006), we acknowledge that they are somewhat subjective.

CONCLUSIONS

Previous research has focused on the effect of PA and ST on CVD and cancer mortality (Nocon et al., 2008; Katzmarzyk et al., 2009; Wilmot et al., 2012), but the effect of these lifestyle factors on mortality from other diseases with a major inflammatory component in older adults has been underexplored. In our study, PA and ST were independently associated with inflammatory mortality other than CVD or cancer in older adults. Also, the highest mortality risk for this cause was observed among older adults with low PA and high ST. Future public health recommendations and clinical interventions should note that addressing both behaviors could have greater benefits on inflammatory mortality than focusing on only one of them. However, our results should be confirmed using objective assessments of PA and ST.

AUTHOR CONTRIBUTIONS

PG-C, EG-E, and FR-A study concept and design. PG-C, EG-E, and FR-A acquisition of data. VC-S, SH-F, and DM-G analysis and interpretation of data. VC-S and DM-G drafting of the manuscript. VC-S, PG-C, SH-F, EG-E, FR-A, and DM-G critical revision of the manuscript for important intellectual content. VC-S, PG-C, SH-F, EG-E, FR-A, and DM-G final approval of the version to be published. VC-S, PG-C, SH-F, EG-E, FR-A, and DM-G agreement to be accountable for all aspects of the work.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphys.2018.00898/full#supplementary-material

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Linear Decrease in Athletic Performance During the Human Life Span

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Master athletes maintain high physical activity levels and have better health than age-matched non-athletes. World records show accelerated declines after age 70 in swimming, long-distance running and sprint performance. However, less is known about age-related performance declines in the general master athlete population and whether decline rates differ between disciplines and genders. We interrogated a dataset including all track and field athletes of North Rhine from 2001 to 2014 to assess age-related changes in performance. 27,088 results of athletes between 11 and 89 years of age in 12 disciplines were analyzed by regression statistics. The analyses showed an accelerated decline beyond the age of 70 in sprint, middle- and long-distance running, while in throwing and jumping disciplines the performance continued a linear decline. Patterns of decline differed between men and women. The steepest declines were observed in javelin throw and 400 m (women), and in pole vault and 800 m (men). In conclusion, performance declines in aging depend more on the specific profile of requirements than previously assumed.

Keywords: athletics, performance, aging, throwing, jumping, sprinting, running, frailty

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INTRODUCTION

The "European Innovation Partnership on Active and Healthy Aging" predicts an increase in the number of people aged 65^{+} in the EU from 85 million in 2008 to 151 million in 2060[1]. An increased ratio of older people dependent on the healthcare system versus the working population contributing to the healthcare system is projected from 28% in 2014 to 50% in 2060 in the Western world (Booth and Hawley, 2015). While the lifespan has increased steadily, this is not the case for the healthspan (McPhee et al., 2016). Part of the problem of the disparity between increased lifespan and healthspan maybe the consequence of low physical activity levels (McPhee et al., 2016) and increased sedentary behavior in the older person (Wullems et al., 2016). Master athletes, on the other hand, maintain high levels of physical activity and retain a better health than age-matched non-athletes (Kettunen et al., 2006) and thus provide a model for the best attainable trajectory of aging (Lazarus and Harridge, 2017).

Track and field athletics is one of the oldest sports and includes running, sprinting, throwing and jumping events. Track and field athletics is popular, and people of all ages participate in competitions. Although world records give a rough understanding of the age-related declines in

[1]https://ec.europa.eu/eip/ageing/

performance (Baker and Tang, 2010; Knechtle and Nikolaidis, 2017), they only reflect performance of the most exceptional individuals (Cheng et al., 2016). A cross-sectional analysis of the performance of master athletes irrespective of their ranking would reveal age-related changes in active individuals in general.

Boccia et al. (2017) published a longitudinal analysis of athletes up to the age of 35 to predict top-level careers in long and high jumpers from plots of individual trajectories of performance against age. Knechtle and Nikolaidis (2017) found in a cross-sectional study that ultramarathon performance peaks between 20 and 35 years of age and is followed by an accelerated decline after the age of 75 years. As each event has its specific requirements regarding speed, power, endurance, agility, coordination etcetera, the rates and patterns of decline may differ between different track and field events. Nevertheless, world records show accelerated declines after the age of 70 in as diverse disciplines as swimming and sprint (Berthelot et al., 2012; Korhonen et al., 2015), suggesting the age-related decline in performance is a general phenomenon, affecting all systems similarly. However, again, this is based on world records, and until now no studies systematically compare trajectories of the age-related decline in performance between disciplines. For a better understanding of the aging process, an analysis of aging-related trajectories of performance decline in several disciplines, irrespective of athletic standing, would be desirable. Patterns of decline would show the maximal compression of morbidity possible by exercise (Lazarus and Harridge, 2017), which maybe particularly important in women who have a lower muscle mass than men, and age-related muscle wasting may thus cause them to cross the disability threshold earlier (Degens and McPhee, 2013). It remains to be seen, however, whether differences in the age-related rates of decline in track and field athletics performance exist between male and female master athletes.

To address these questions, we interrogated a large data set including all registered athletes of North Rhine in Germany over many years to assess the trajectory of age-related changes in performance in several athletic disciplines. The hypotheses were: (1) the pattern of decline does not differ between events and (2) between men and women, (3) there are no differences in peak performance age between disciplines and genders, while (4) a more rapid decline in performance occurs after the age of 70. We also had the opportunity to analyze longitudinal changes in performance in several athletes who competed over many years and hypothesized no differences between longitudinal and cross-sectional changes in performance.

MATERIALS AND METHODS

Ethical approval was obtained from RWTH Aachen University Hospital IRB (reference number EK 300/17, date of approval: October 11, 2017).

Generation of Data-Set and Data Availability

Athlete performance data was extracted from the official rankings lists of annual best results of each discipline in 2001–2014 of North Rhine Track and Field Association. The datasets analyzed for this study can be found in the result list repository of LV Nordrhein and are publicly available in html-format under the following URL[2]. North Rhine is a part of Germany and has a total population of approximately 17.5 million. Data was extracted from html-files and automatically reformatted into a table of individual athletes' year-by-year (absolute age) best performance values for each discipline, using a script written in Perl (**Supplementary File**). Data was sorted for analysis using the same script and times were re-formatted into seconds.

Statistical Analysis

Each year's result lists comprise the best 20 results in each age group in each discipline. In master athletes, due to low participation, there are always fewer than 20 athletes in the lists, which means everyone who participated showed up in the ranking list. In non-master athletes, the age groups were: 11, 12, 13, 14, 15, 16/17, 18/19, and 20–29 years of age (men/women = the main class). In Germany, master classes already start at age 30, while internationally they begin at 35. Master classes continue in 5-year categories for both women and men (30–34, 35–39 etc.).

In the non-master athlete groups, athletes only show up when they exceed a performance threshold, which means only the best appear in the rankings. This explains the jump decrease in performance between 29 and 30 years of age, visible in many of the graphs. Due to this phenomenon, we decided to perform regression analyses for athletes younger than 20 and older than 29, and to ignore the performance data of people between 20 and 29 years of age in the regression analyses. Nevertheless, we do show the 20–29-year data in the graphs. Linear regression was found to deliver the highest R^2, indicating the best match compared to exponential, logarithmic and polynomic regression. A second regression analysis was conducted to analyze a possible accelerated decline beyond age 70. Here, data was normalized to performance at age 30. Again, linear regression showed higher R^2 than non-linear regression. Performance decline in percent per year was computed for athletes between 30 and 69 years and those 70 years and older. An accelerated decline after the age of 70 was assumed if the difference in the slope of the regression line was larger than 0.25 (25% difference). Likewise, differences in the slope of the regression line larger than 0.25 between disciplines or genders reflected a different age-related rate of decline between disciplines or genders, respectively. In addition, a third regression analysis was done for athletes under 30 years and athletes between 30 and 69 years to calculate peak performance age for each discipline and gender. The following formula was used (regression equation: $Y = aX + b$): peak performance = $(b < 30 – b > 30)/(a > 30 – a < 30)$.

RESULTS

A total of 27,088 results of athletes between 11 and 89 years of age were included in the analysis. **Table 1** shows the data distribution

[2]http://archiv.lvnordrhein.de/index.php/wettkaempfe/bestenlisten/lvn-bestenlisten

TABLE 1 | Numbers of results in each event.

	F	M	Total
100 m	1,040	1,834	2,874
200 m	699	1,212	1,911
400 m	576	1,033	1,609
800 m	964	1,362	2,326
1,500 m	576	1,155	1,731
5,000 m	689	1,590	2,279
Shot put	1,113	1,875	2,988
Javelin throw	881	1,591	2,472
Discus throw	1,006	1,782	2,788
Long jump	1,004	1,651	2,655
High jump	821	1,386	2,207
Pole vault	347	901	1,248
Sum:	9,716	17,372	27,088

F, female; M, male.

over the different disciplines and between genders. Almost twice as many results are available from male (17,372) than female athletes (9,716). **Figures 1–4** display results of the regression analysis for sprints (**Figure 1**), middle and long-distance runs (**Figure 2**), jumps (**Figure 3**), and throws (**Figure 4**). The graphs show the regression equations for performance vs. age between 11 and 20, and 30–70-years, separated for men and women.

Participation in Different Disciplines

The most popular disciplines are 100 m, shot put, discus throw and long jump (**Table 1**). In the 400 m sprint (**Figure 1**), athlete numbers decreased after the age of 55 and in pole vault (**Figure 3**) very few athletes continue beyond age 30. **Figure 4** shows a transient reduction in participation in the throwing disciplines between 30 and 40 years of age, while an increase in participants occurs after the age of 60 in men but not in women. Disciplines with the oldest athletes appearing in the data set are shot put and discus throw (maximum age: 89 years).

Age-Related Changes in Performance

Figures 1–4 show a maturational increase in performance in all events followed by an age-related decline in performance. Results indicate the least variation in long jump where R^2 is highest (men: 0.703, women: 0.598; **Figure 3**). In running disciplines, an accelerated decline beyond the age of 70 was found (**Table 2**). In the throwing and jumping disciplines, however, no such accelerated decline is present. The only exception was for the 200 m women, where the difference is not significant, probably because only 21 athletes were older than 69 years.

Differences in Age-Related Decrements in Performance Between Genders and Disciplines

The patterns of decline appear to differ between genders, as reflected by the differences in the annual percentage declines in performance in men and women (**Tables 2**, **3** and **Figure 5**). Performance shows the least steep decline in discus throw and 5,000 m running in women and in shot put and 100 m in men (**Table 2**). In men between 30 and 69 years of age, pole vault, 400, and 800 m show the steepest declines. In women, javelin throw, 400 and 800 m and discus throw decline fastest. We therefore analyzed javelin throw and pole vault further, applying an additional analysis to compare longitudinal and cross-sectional results. **Figure 6** shows longitudinal changes in javelin throwing performance of those individual athletes who appear at least with seven results in our data-set. Their declines follow the same pattern as observed in the cross-sectional data of the corresponding discipline in **Figure 4**. Student's *t*-test revealed no significant difference between cross-sectional and longitudinal data ($p = 0.254$). While pole vault has the steepest decline of all events in men, it showed the slowest decline in women. **Figure 6** also shows a comparison of cross-sectional and longitudinal data for pole vault. All results in the women older than 45 years originate from the same athlete, indicating that the slower decline in pole vault performance in women needs to be interpreted with caution.

Results of the calculation of peak performance age are shown in **Table 4**. The discipline 400 m shows the highest age of peak performance in both genders. On average women peak approximately 2 years later than men (21.6 years vs. 19.7 years).

DISCUSSION

In the present study, a dataset of athletics results was created from 14 annual ranking lists of North Rhine, Germany. In total, 27,088 results from athletes of all age groups in 12 disciplines were analyzed. The main findings are: (1) the age-related decline in performance accelerates after the age of 70 in the sprint and running disciplines, but not in throwing and jumping disciplines; (2) patterns of decline differ between events and (3) between men and women; (4) there are differences in peak performance age between disciplines and genders; (5) no significant differences were found between longitudinal and cross-sectional data.

Patterns of Performance Decline

The performance in all disciplines showed a linear decline up to the age of 70 years. This is identical to the linear decline in $\dot{V}O_2$max (Tanaka and Seals, 2008) and power (Pearson et al., 2002) in master endurance and power lifter athletes that thus may underlie the age-related decline in performance we observed here. After the age of 70 years, however, the decline in the sprint and running but not in the throwing and jumping disciplines was accelerated, in accordance with results published previously (Tanaka and Seals, 2008; Rittweger et al., 2009; Baker and Tang, 2010; Lazarus and Harridge, 2017). In a review article on endurance exercise performance in master athletes, Tanaka and Seals (2008) describe progressively steeper declines at high age in marathon and swimming. Rittweger et al. (2009) analyzed master world records showing accelerated declines after age 70 in long distance, but less so in sprint disciplines. Baker and Tang (2010) compared performance declines in running, cycling, swimming, weightlifting, rowing, triathlon,

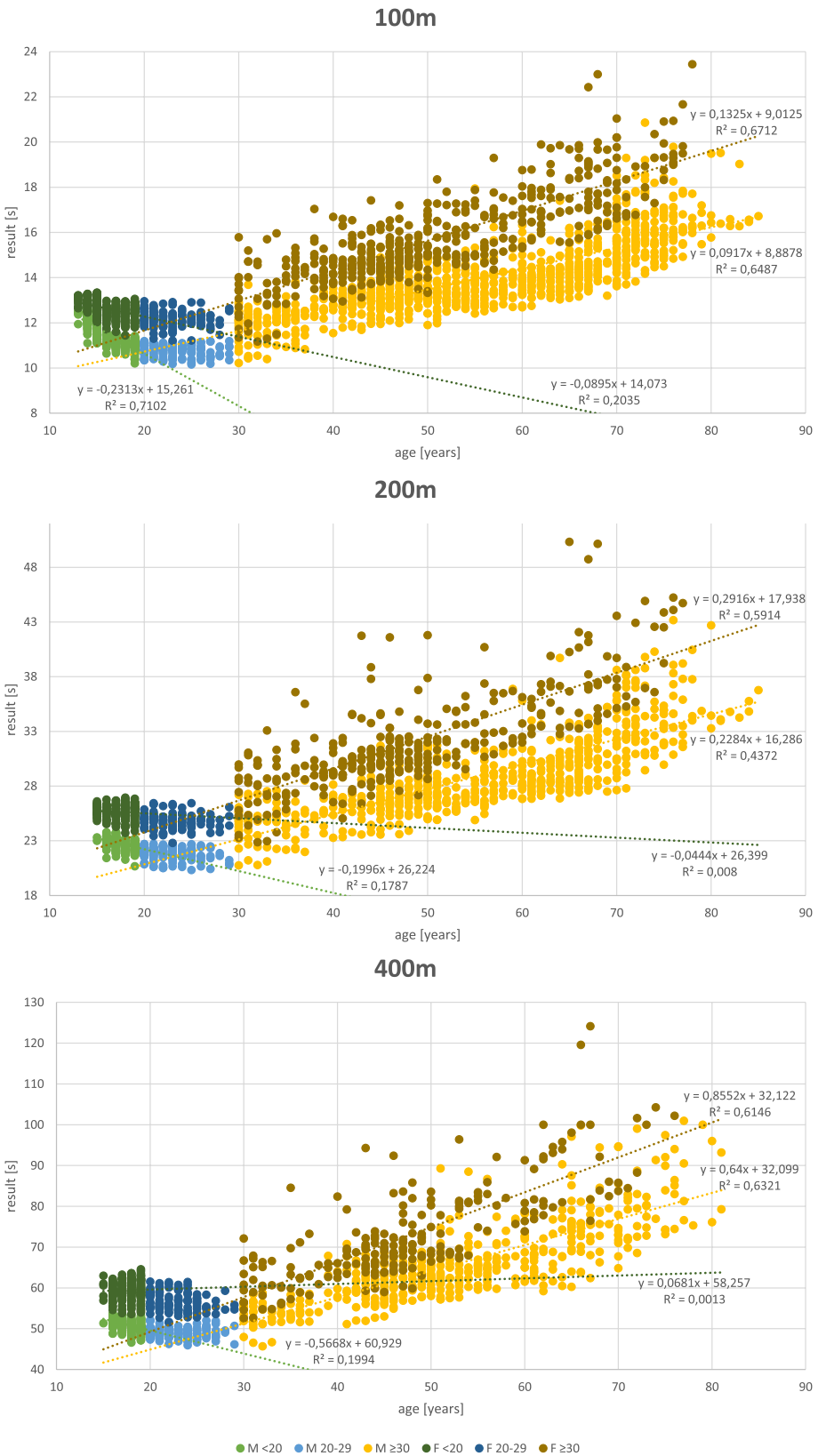

FIGURE 1 | Regression analysis for the sprint disciplines (100, 200, and 400 m).

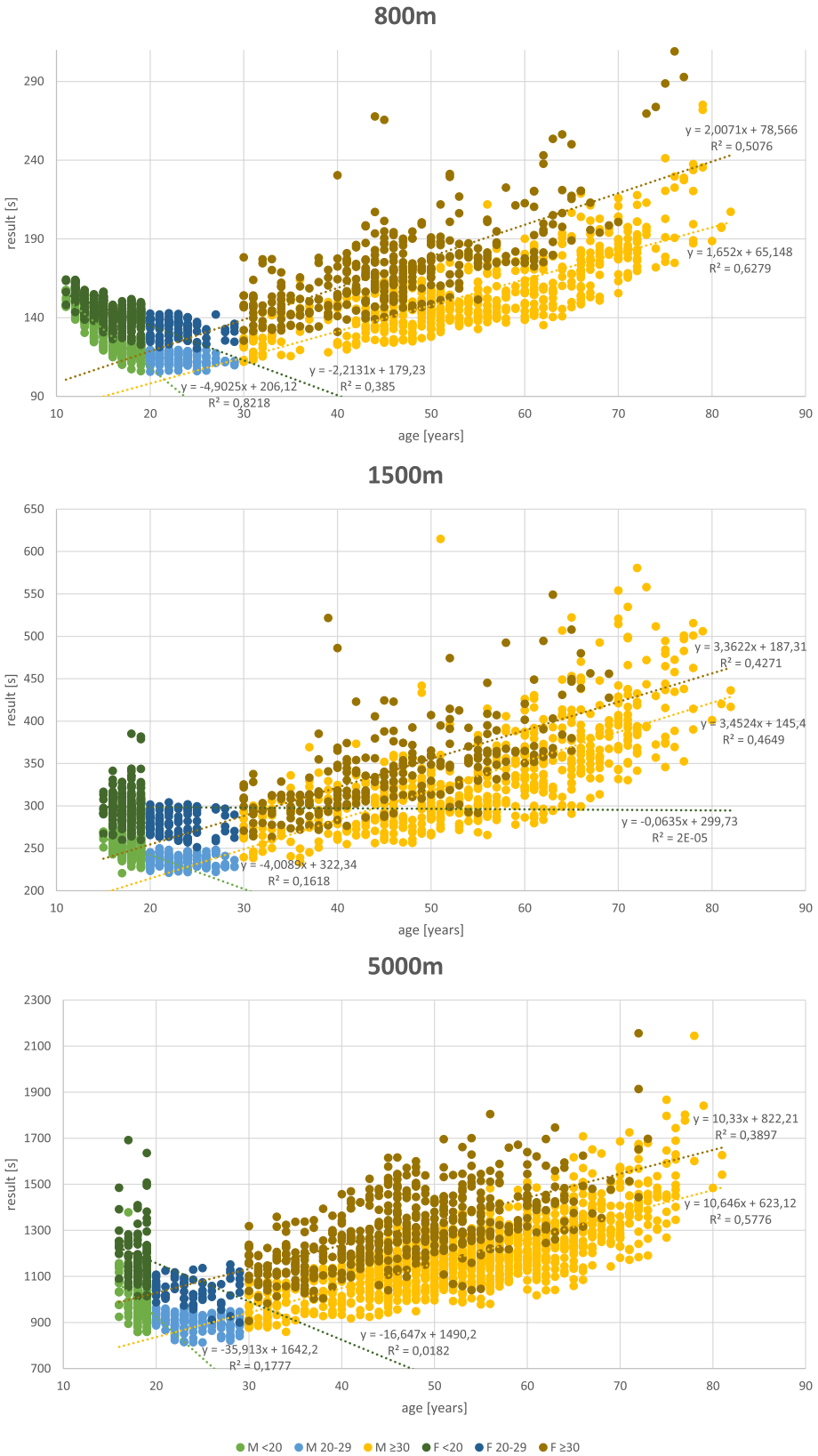

FIGURE 2 | Regression analysis for middle and long-distance running (800, 1,500, and 5,000 m).

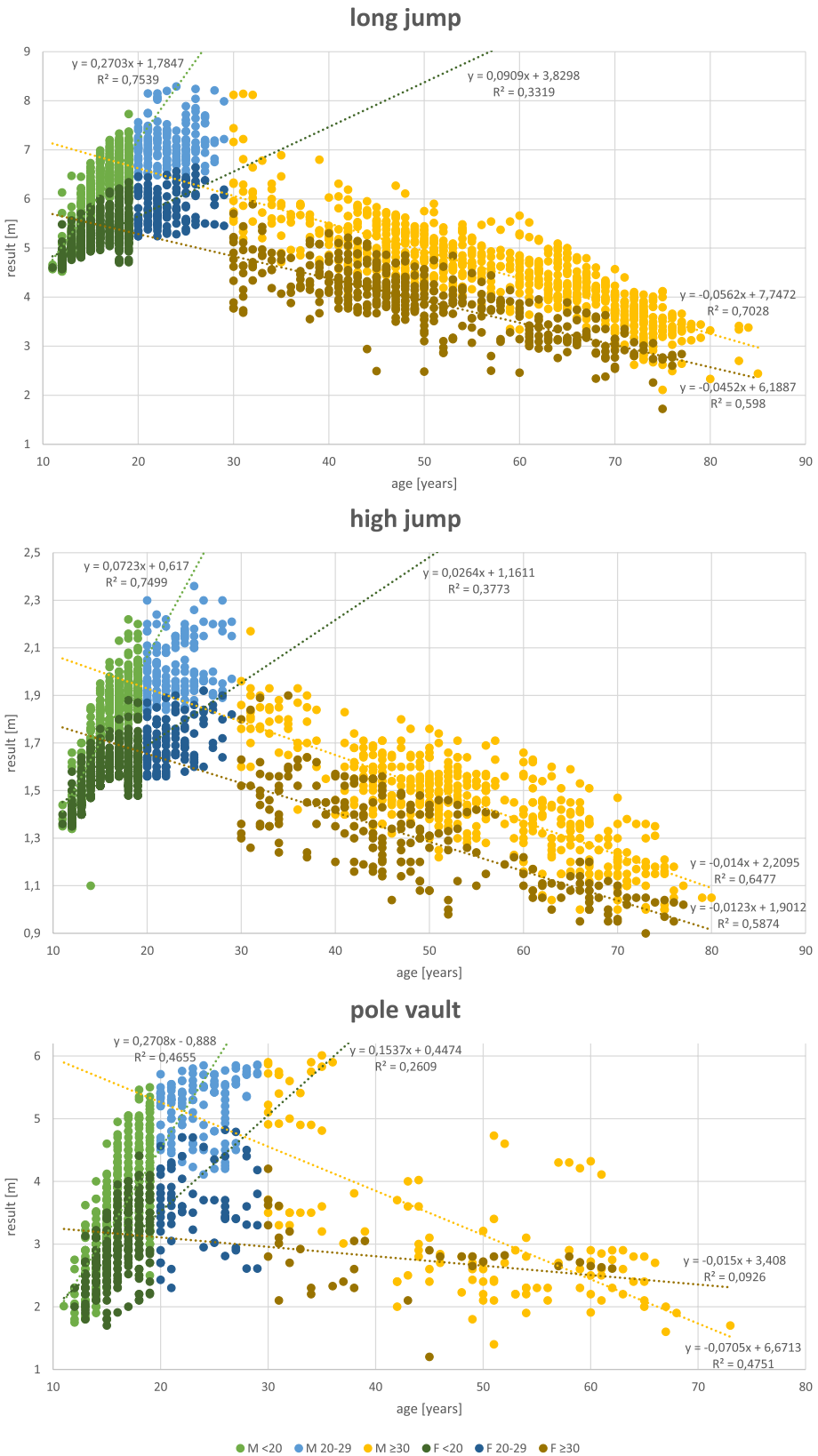

FIGURE 3 | Regression analysis for the jumps (long jump, high jump, and pole vault).

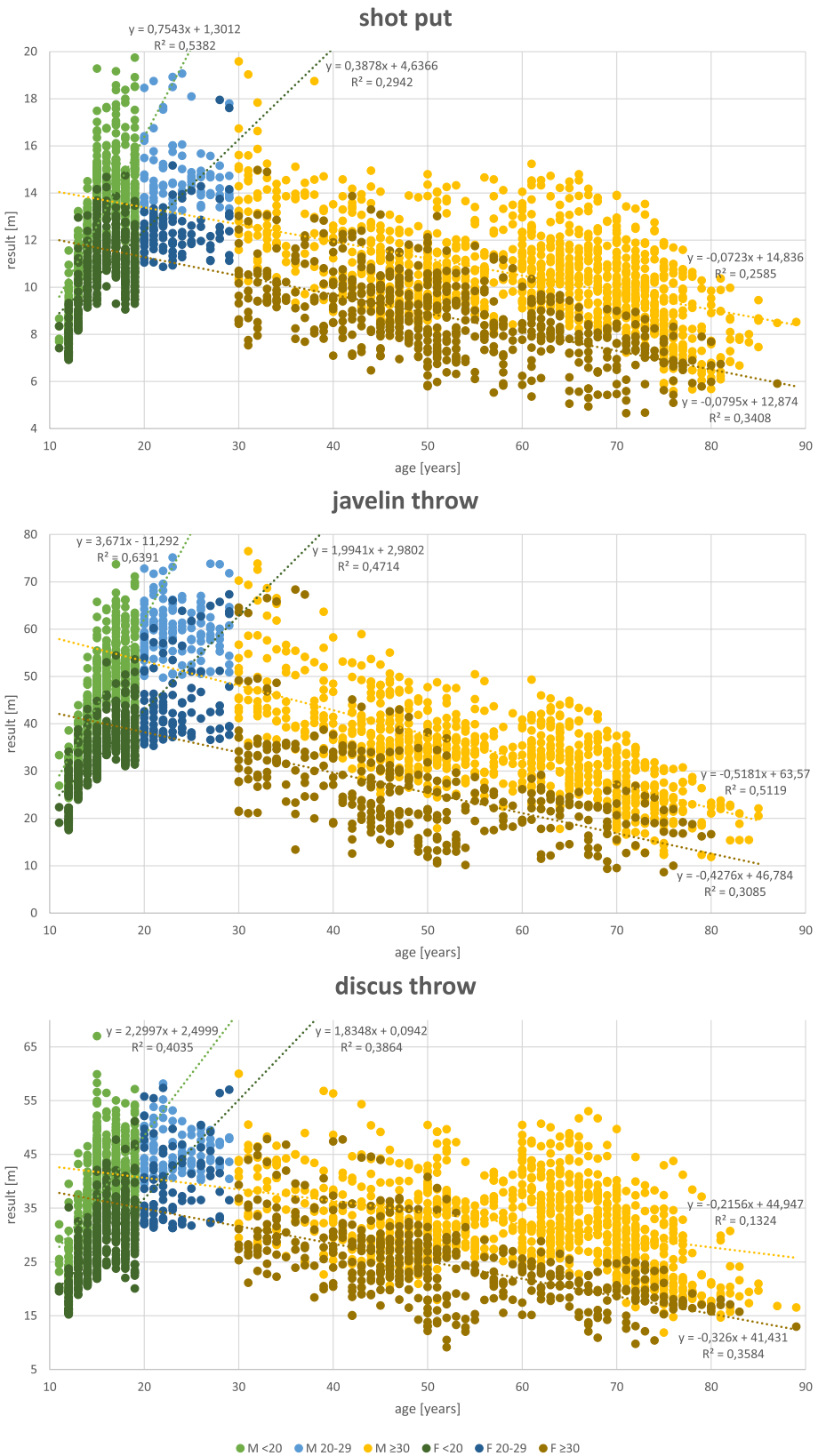

FIGURE 4 | Regression analysis for the throws (shot put, javelin throw, and discus throw).

TABLE 2 | Analysis for accelerated declines beyond the age of 70 and differences in the rate of decline in performance between 30 and 70-year-old male and female athletes.

Discipline	F < 70	F ≥ 70	Δ	% Δ	sign.	M < 70	M ≥ 70	Δ	% Δ	sign.	$\Delta_{F,M}$ < 70	% $\Delta_{F,M}$ < 70	Sign.
100 m	0.959	2.239	1.276	133	Yes	0.581	1.001	0.420	72	Yes	0.378	39	Yes
200 m	1.022	1.115	0.094	9	No	0.680	1.388	0.709	104	Yes	0.342	33	Yes
400 m	1.415	5.972	4.558	322	Yes	1.072	2.153	1.081	101	Yes	0.343	24	No
800 m	1.171	9.629	8.459	723	Yes	1.077	3.036	1.959	182	Yes	0.094	8	No
1,500 m	1.100	n/a	n/a			1.021	1.343	0.323	32	Yes	0.079	7	No
5,000 m	0.838	7.892	7.054	842	Yes	0.939	3.103	2.164	230	Yes	0.101	11	No
Shot put	0.788	0.693	−0.096	−12	No	0.363	1.503	1.140	314	Yes	0.425	54	Yes
Javelin throw	1.688	0.929	−0.759	−45	No	0.950	1.102	0.152	16	No	0.738	44	Yes
Discus throw	1.182	0.958	−0.224	−19	No	0.015	1.825	1.811	12,318	Yes	1.167	99	Yes
Long jump	0.944	1.144	0.2	21	No	0.752	0.857	0.104	14	No	0.192	20	No
High jump	0.843	0.256	−0.587	−70	No	0.729	0.701	−0.028	−4	No	0.114	14	No
Pole vault	0.427	n/a	n/a			1.419	n/a	n/a			0.992	70	Yes

Decline in percent per year normalized to the average result at age 30. Significance was assumed at 25%. A negative % shows a slower decline in performance after the age of 70. F, female; M, male; Δ, difference. %Δ, difference from initial decline; dark gray cells show a slowing of the age-related decline; %ΔF,M < 70, %difference from the gender with the highest slope; dark gray cells, men faster decline than women; light gray cells, women faster decline than men.

TABLE 3 | Analysis of differences in rate of performance decline in percent per year (normalized to 30 years) between disciplines (upper number) and the delta/steepest slope of the pair (lower number) in 30- to 70-year-old athletes.

	100 m	200 m	400 m	800 m	1,500 m	5,000 m	Shot put	Javelin	Discus	Long jump	High jump	Pole vault
100 m		0.099	0.491	0.496	0.440	0.358	0.218	0.370	0.566	0.172	0.148	0.838
		14.5%	45.8%	46.1%	43.1%	38.1%	37.5%	38.9%	97.4%	22.8%	20.3%	59.1%
200 m	0.063		0.392%	0.397%	0.341	0.260	0.317	0.271	0.665	0.073	0.050	0.739
	6.2%		36.5%	36.9%	33.3%	27.7%	46.6%	28.5%	97.8%	9.7%	6.9%	52.1%
400 m	0.456	0.393		0.005	0.051	0.132	0.709	0.121	1.057	0.319	0.342	0.347
	32.2%	27.8%		0.5%	4.8%	12.3%	66.1%	11.3%	98.6%	29.8%	31.9%	24.5%
800 m	0.212	0.149	0.244		0.056	0.138	0.714	0.127	1.062	0.325	0.348	0.342
	18.1%	12.7%	17.2%		5.1%	12.8%	66.2%	11.8%	98.6%	30.2%	32.3%	24.1%
1,500 m	0.141	0.078	0.315	0.071		0.081	0.658	0.070	1.006	0.268	0.291	0.398
	12.8%	7.1%	22.2%	6.1%		7.9%	64.4%	6.9%	98.5%	26.2%	28.5%	28.0%
5,000 m	0.120	0.183	0.577	0.332	0.261		0.577	0.011	0.925	0.187	0.210	0.479
	10.9%	17.9%	40.8%	28.4%	23.7%		61.4%	1.2%	98.5%	19.9%	22.4%	33.8%
Shot put	0.171	0.234	0.627	0.383	0.312	0.050		0.588	0.348	0.390	0.367	1.056
	17.8%	22.8%	44.3%	32.7%	28.4%	6.0%		61.8%	95.8%	51.9%	50.3%	74.4%
Javelin	0.729	0.666	0.273	0.517	0.588	0.849	0.900		0.936	0.198	0.221	0.468
	43.2%	39.5%	16.1%	30.6%	34.8%	50.3%	53.3%		98.5%	20.8%	23.3%	33.0%
Discus	0.223	0.160	0.233	0.011	0.082	0.343	0.394	0.506		0.738	0.715	1.404
	18.9%	13.5%	16.5%	1.0%	6.9%	29.0%	33.3%	30.0%		98.1%	98.1%	98.9%
Long jump	0.014	0.077	0.470	0.226	0.155	0.106	0.156	0.743	0.237		0.023	0.666
	1.5%	7.5%	33.2%	19.2%	14.1%	11.2%	16.5%	44.0%	20.1%		3.1%	46.9%
High jump	0.115	0.178	0.571	0.327	0.256	0.005	0.055	0.844	0.338	0.101		0.689
	12.0%	17.4%	40.4%	27.9%	23.3%	0.6%	6.5%	50.0%	28.5%	10.7%		48.6%
Pole vault	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	

Left/bottom, women; right/top, men. The % difference was calculated as the difference in slope between disciplines divided by the steepest slope. Dark gray indicates rate of decline in performance in parameter column larger than in the parameter in the row; light gray indicates decline in the performance of the parameter in the row declines faster than the performance of the parameter in the column.

walking and jumping, and assumed accelerated "curvilinear" declines with age for all these sports. We found an accelerated performance decline after age 69 in an analysis of a small group of male master javelin throwes (Ganse and Degens, in press).

In the throwing disciplines, such an accelerated decline in performance was not always seen. Part of the cause of the absence of an accelerated decline in performance in the throwing disciplines is that the implemented weights decrease over the years. In men, the last decrease of implement weights takes place at the age of 80 in shot put and javelin throw, and at 60 in discus throw. In women, at age 75 the last weight change occurs in shot put, discus and javelin throw. The implication for our analysis

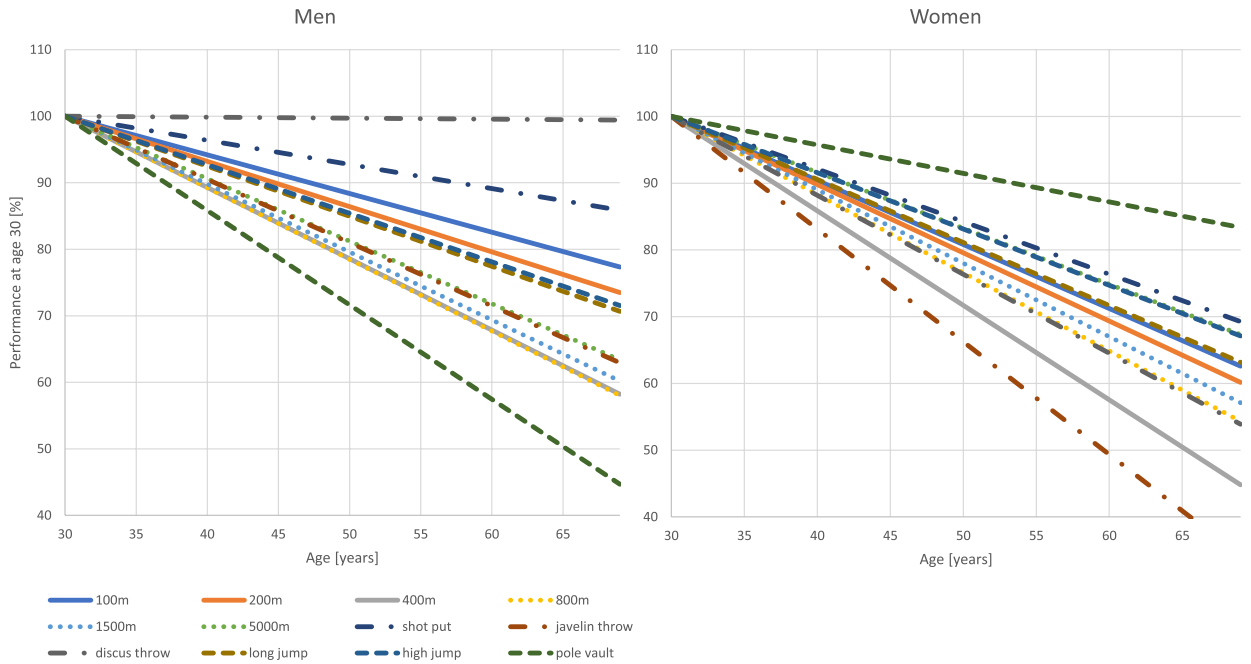

FIGURE 5 | Comparison of regression lines of athletes 30–69 years in all disciplines and for both genders normalized to performance at 30 years (=100%).

is that a similar distance thrown in older age in reality reflects a lower performance than a similar distance thrown at a younger age. Even so, also in the men's shot put and discus throw there was an accelerated decline in performance after the age of 70, despite the decrease in the mass of the discus and put thrown. While the increase in numbers of athletes in these disciplines after retirement (Engberg et al., 2012; Schönbach et al., 2017) will result in an increased proportion of less-performing athletes that undoubtedly accentuate the population-based accelerated decline in performance after the age of 70, in the running disciplines participation numbers are not showing a large increase after the age of 70 (**Figures 1**, **2**). Thus, overall there appears to be an accelerated decline in performance after the age of 70 years. Lazarus and Harridge (2017) suggest that this pattern reflects a trajectory of a "fading integrative physiological capacity." If the progressive age-related decline in bodily functions is a stochastic process, as seen in the accumulation of DNA damage (Cortopassi and Wang, 1996), then indeed it is to be expected that the decline in performance accelerates in old age (Degens, 2012).

As discussed above, the rate of decline was fastest in longer sprints and middle distances with relatively slow declines in throwing disciplines. Overall, our results indicate slower declines in anaerobic disciplines compared to aerobic disciplines. These results differ from those of Baker and Tang (2010) who compared performance declines in running, cycling, swimming, weightlifting, rowing, triathlon, walking and jumping, and found the fastest declines in weightlifting. Similarly, Gent and Norton (2013) found that in master cyclists in peak the anaerobic performance declined more than aerobic performance. Weightlifting is related to muscle power and should therefore closely compare to the throwing disciplines, especially shot put. However, in our data, shot put was the discipline with the least steep decline in men and an average rate of decline in women. A possible explanation for the different results might be the complexity of track and field disciplines that always require a mix of speed, agility, power and other factors. Another possible explanation might be the specific training track and field athletes undergo focussing on their disciplines, while Gent and Norton (2013) studied cyclists and tested them in ways that do not follow their usual training pattern. Baker and Tang (2010), however, studied results of the actual competition just like in our study.

Part of the discrepancy between the study by Baker and Tang (2010) and our study may be attributable to the different disciplines compared in theirs and our study suggesting part of the differences in the age-related rate of performance decline is attributable to factors other than changes in physiology, such as techniques, that modulate the age-related decline in performance. For instance, javelin throw shows the fastest decline of all throws and unlike other throwing events, not only arm, upper body and core strength are required, but also speed and agility (Kunz and Kaufmann, 1983). Pole vault, on the other hand, shows the fastest decline of all disciplines in men and is not only technically highly complex and demanding, but also requires the most diverse and extensive training (Linthorne and Weetman, 2012). While some studies have shown a faster decline in anaerobic power events and jumping than in aerobic and running events (Baker and Tang, 2010; Gent and Norton, 2013) we could not make such a clear distinction. It should be noted that Baker and Tang (2010) also found that some endurance events, such as cycling, and triathlon declined faster than the running and swimming performance. It

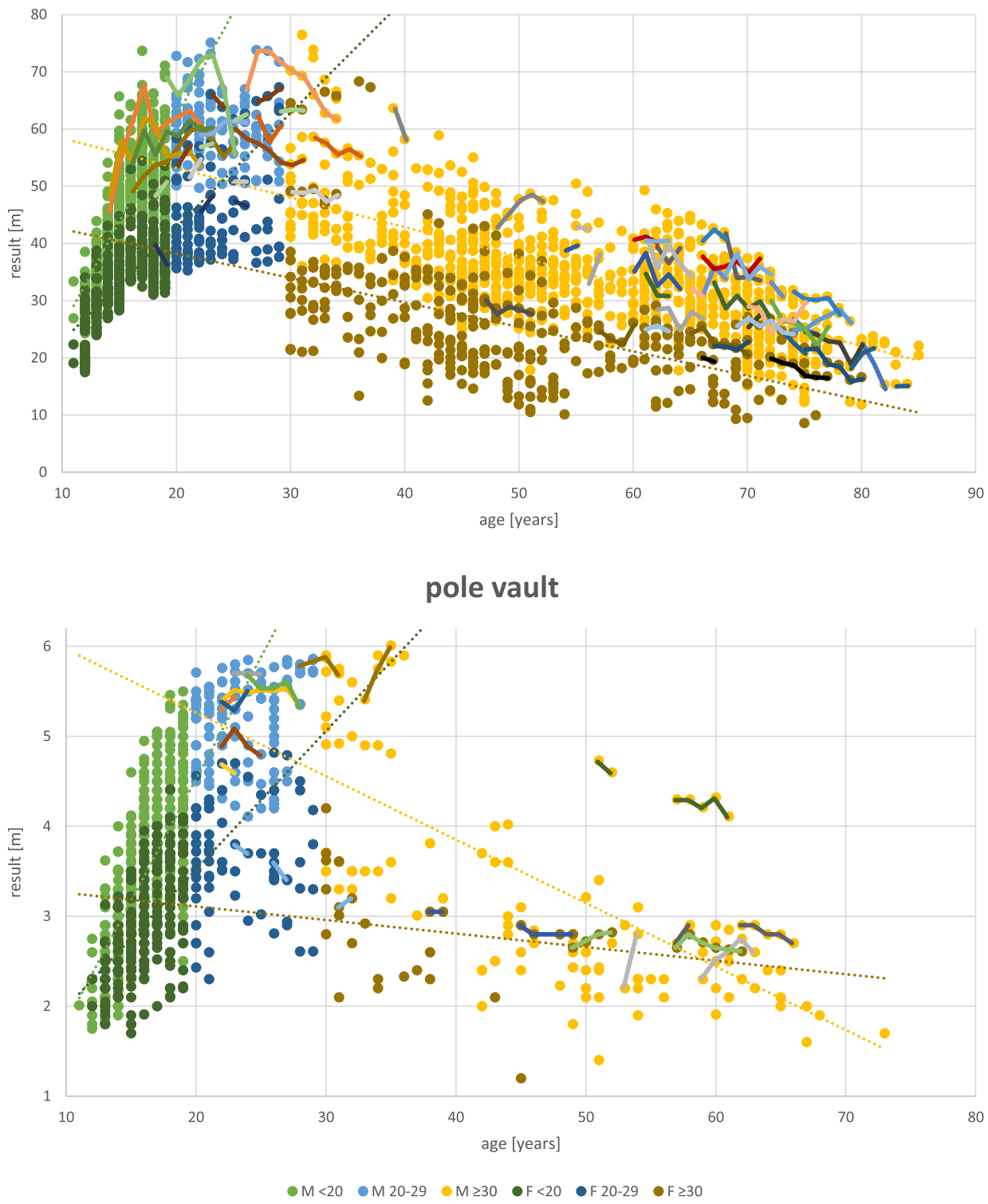

FIGURE 6 | Overlay of the javelin throw and pole vault plots with curves of all athletes who have seven or more results in this data set.

does seem that not only strength or metabolism, but also changes in technique contribute to the age-related decline in performance.

Gender Aspects

Gender differences in declines of track and field disciplines have to our knowledge not previously been reported. Although in our data set we had twice as many results of men compared to women and men competed to higher age, we still had a substantial set of data from female master athletes. Explanations for the lower participation of women are mainly cultural and relate to traditional role models and socio-economic status (Seabra et al., 2008; Toftegaard-Støckel et al., 2011). While male participation in the throwing disciplines increased after the age of 60, most likely due to retirement, this increase was not observed in women. Reasons could be the lack of an abrupt retirement wave of women in Germany, who work in general fewer hours and retire earlier than men (Leve et al., 2009). Another aspect for the lower participation of women is that while the first athletics competitions for women at Olympic Summer Games took place in 1928, most disciplines were only performed by women much later. The women's long jump, 200 m and shot put showed up in 1948, 400 m in 1964, 5,000 m in 1996 and pole vault as late as 2000 (Schade et al., 2004). Nevertheless, there were no massive overall differences in the age-related decline in performance between genders, but rather varieties in detail. It is doubtful whether these differences in detail are real, as the lower numbers of female participants can probably explain most of gender-related differences in the rate of age-related decline in performance. Future studies are needed to confirm whether gender-related differences in the age-related decline in performance really exist.

Peak Performance Age

The calculated peak performance age from regression data can only be rough estimates, especially because peak performance in elite athletes is to some degree determined by training intensities and support by clubs, family and sport organizations (Allen et al., 2015). In running, the age of peak performance has been reported to increase with race distance (Knechtle and Nikolaidis, 2018) and was estimated to be between 39 and 41 years in marathon and ultramarathon runners (Nikolaidis and Knechtle, 2018). In our analysis, we found much earlier peak performance in track and field disciplines, similar to the age of peak performance between 25 and 27 years for those disciplines observed before (Haugen et al., 2018). Haugen et al. (2018) observed a higher peak performance age in marathon runners and male throwers. Interestingly, similar to the observation by Haugen et al. (2018) we found that the age of peak performance was in general higher for women than men. However, if our calculations are correct, then the age of peak performance with regards to the disciplines analyzed in the present study (maximum 5,000 m) was highest for the 400 m sprint and not, as expected from the previous studies, in the longer distances. The discrepancy may be attributable to the fact that we included all athletes, while other studies only compared peak performers, and that we extrapolated the data. Whatever the cause of this discrepancy, the implication for coaches and officials is that talents in the long sprints and middle-distance disciplines need support for a longer period to reach their personal best than in other disciplines, such as in the jumps. High peak performance age also means collision with family planning and working life, and athletes in sports that peak late need more support in these areas if we want them to continuously perform well on international scale.

TABLE 4 | Peak performance age calculated from regression equations.

	Peak F	Peak M
100 m	22.86	18.78
200 m	22.83	19.53
400 m	27.15	23.91
800 m	23.63	22.39
1,500 m	26.06	22.43
5,000 m	23.41	23.75
Shot put	18.61	15.75
Discus throw	19.70	13.06
Javelin throw	19.60	18.85
Long jump	17.21	18.57
High jump	19.92	19.54
Pole vault	18.23	23.09
Average	21.60	19.97

F, female; M, male.

Psychological Aspects

Apart from age-related decrements in physiological function, motivational changes across the athletic lifespan may also contribute to the age-related decline in performance (Medic et al., 2007). The authors showed that athletes are usually able to break records during the first 2 years in a 5-year age group and participation drops in the latter half of the 5-year age category, reflecting the influence of psychology at least on participation.

Limitations

Our study is the first to analyze a large dataset regarding declines in performance of athletes in several athletics disciplines. The major strength of the study is the large amount of data. Though the data presented here is primarily of cross-sectional nature, the longitudinal data contained in the dataset follows the same pattern of decline as seen in the cross-sectional analysis. This indicates that cross-sectional analyses provide a good reflection of the age-related declines in athletic performance, and cross-sectional analyses may thus also give a good indication of age-related changes in other measures, such as muscle strength and maximal oxygen consumption. A potential weakness is that there were no athletes older than 89 in the dataset, precluding any firm conclusions on performance changes in the oldest-old master athletes.

CONCLUSION

In conclusion, performance declines accelerated beyond the age of 70, particularly in runners and sprinters, while the real

pattern of decline might be hidden in throwers due to decreasing implement weights. As age-related rates of decline differ between disciplines it indicates that the decline in performance is complex and dependent on both changes in physiology and technique. Significantly, the age-related decline seen in the few athletes whom we could follow longitudinally followed a similar time course compared to the cross-sectional data. The implication is that population wide cross-sectional studies give a good indication of the age-related changes in performance and physiology of athletes. Of course, individual factors such as comorbidities, genetics and lifestyle play major roles for individual development. Injuries and disease may stop athletes' careers and decrease performance.

AUTHOR CONTRIBUTIONS

BG contributed the idea and worked on data analysis and interpretation, figures, tables, drafting, manuscript submission, and approval of the manuscript. UG worked on data collection, data interpretation, and approved the manuscript. JD worked on data collection and interpretation, and approved the manuscript. HD contributed to the statistical analysis, data discussion, drafting, and approval of the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphys.2018.01100/full#supplementary-material

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ORIGINAL RESEARCH
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Normal Values of Myocardial Deformation Assessed by Cardiovascular Magnetic Resonance Feature Tracking in a Healthy Chinese Population: A Multicenter Study

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Reference values on atrial and ventricular strain from cardiovascular magnetic resonance (CMR) are essential in identifying patients with impaired atrial and ventricular function. However, reference values have not been established for Chinese subjects. One hundred and fifty healthy volunteers (75 Males/75 Females; 18–82 years) were recruited. All underwent CMR scans with images acceptable for further strain analysis. Subjects were stratified by age: Group 1, 18–44 years; Group 2, 45–59 years; Group 3, ≥60 years. Feature tracking of CMR cine imaging was used to obtain left atrial global longitudinal (LA E_{ll}) and circumferential strains (LA E_{cc}) and respective systolic strain rates, left ventricular longitudinal (LV E_{ll}), circumferential (LV E_{cc}) and radial strains (LV E_{rr}) and their respective strain rates, and right ventricular longitudinal strain (RV E_{ll}) and strain rate. LA E_{ll} and LA E_{cc} were 32.8 ± 9.2% and 40.3 ± 13.4%, respectively, and RV E_{ll} was −29.3 ± 6.0%. LV E_{ll}, LV E_{cc} and LV E_{rr} were −22.4 ± 2.9%, −24.3 ± 3.1%, and 79.0 ± 19.4%, respectively. LV E_{ll} and LV E_{cc} were higher in females than males ($P < 0.05$). LA E_{ll}, LA E_{cc}, and LV E_{cc} decreased, while LV E_{rr} increased with age ($P < 0.05$). LV E_{ll} and RV E_{ll} were not shown to be associated with age. Normal ranges for atrial and ventricular strain and strain rates are provided using CMR feature tracking in Chinese subjects.

Keywords: cardiovascular magnetic resonance, feature tracking, strain and deformation, Chinese, multicenter

INTRODUCTION

Assessment of chamber function is an important objective of a cardiac imaging study. In assessing chamber function, myocardial deformation is superior to left ventricular (LV) ejection fraction for prognostication in patients with various myocardial disorders (Stanton et al., 2009; Motoki et al., 2012; Zhong et al., 2013; Kalam et al., 2014). Several advanced techniques based on either echocardiography or cardiovascular magnetic resonance (CMR),

such as real-time speckle-tracking echocardiography (Zhang et al., 2016), tissue tagging and feature tracking (Zhong et al., 2012; Venkatesh et al., 2014; Khan et al., 2015), have become available for assessing myocardial deformation. Advantages of these techniques include excellent scan-rescan reproducibility, less dependence on operator technique, and more accurate and reproducible measures of the left and right ventricles. Consequently, CMR has become the standard reference modality for measurement of ventricular volume and function (Bellenger et al., 2000), and arguably the optimal imaging modality for quantification of myocardial displacement (Leng et al., 2015, 2016, 2018), strain and strain rate (Rahman et al., 2017; Scatteia et al., 2017; Koh et al., 2018; Zhao et al., 2018).

Cardiovascular magnetic resonance feature tracking (CMR-FT) is analogous to speckle-tracking echocardiography and allows quantification of global and regional myocardial motion and deformation using standard, balanced steady state free precession (SSFP)/balanced turbo field echo (BTFE) cine CMR images in long and short axis views, which are imperative in routine clinical CMR practice (Augustine et al., 2013). Reference values of cardiac strain for the Western population have been reported in Andre et al. (2015), Kawel-Boehm et al. (2015), Taylor et al. (2015), Mangion et al. (2016). Distribution patterns of LV myocardial strain in healthy Chinese volunteers were provided using deformation registration algorithm (TrufiStrain, Siemens Healthcare), however, strain analysis for left atrium and right ventricle was not performed (Liu et al., 2017).

In this study, we aimed to establish CMR reference values for left atrial (LA), left ventricular (LV), and right ventricular (RV) strains and strain rate in Chinese subjects, and to evaluate the effects of age and gender on strain and strain rate measurements.

MATERIALS AND METHODS

Population

Healthy volunteers who met the following inclusion criteria were prospectively recruited from two centers in China

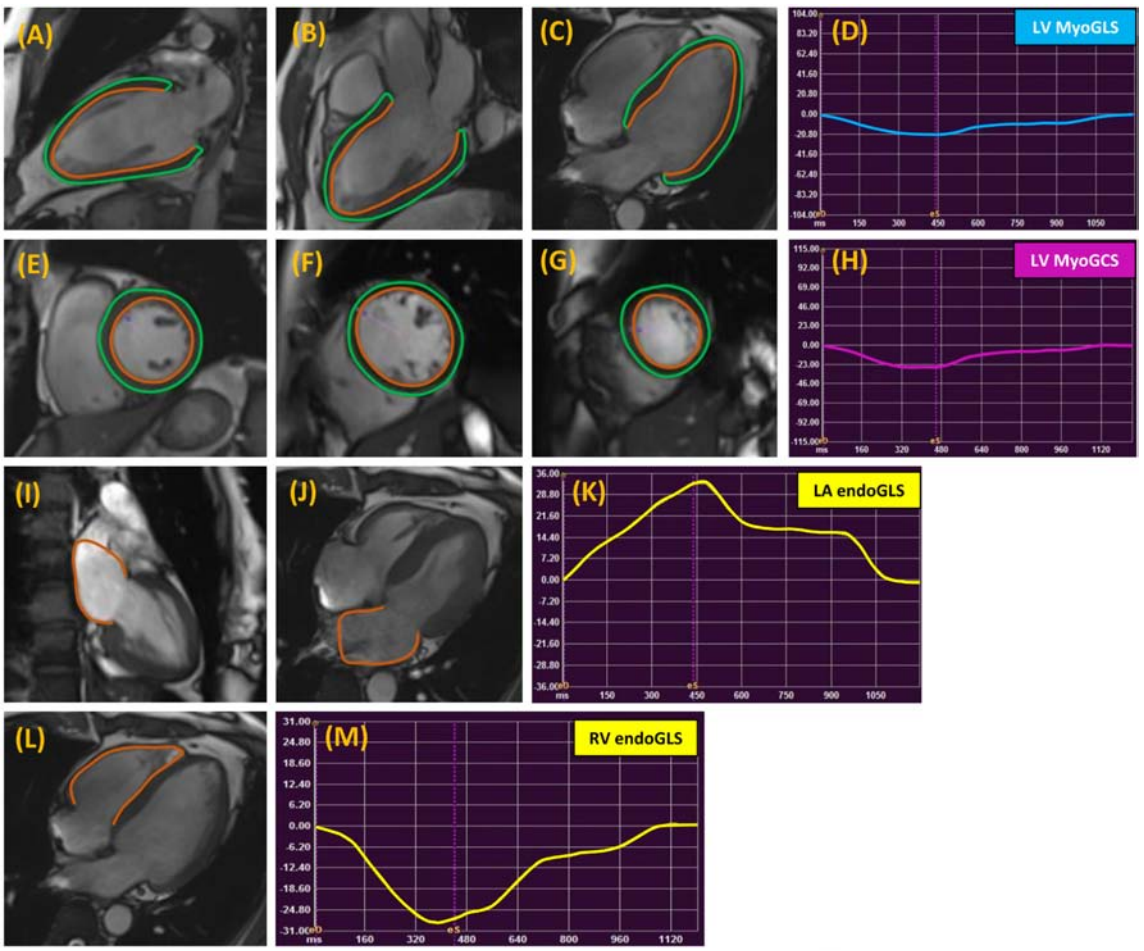

FIGURE 1 | Representative images in the standard long axis **(A–C)** and short axis **(E–G)** orientations and strain curves. Contours are illustrated in left ventricular endocardial and epicardial borders in the 2-, 3-, 4-chamber **(A–C)** and corresponding LV MyoGLS (LV E_{ll}) **(D)**; endocardial and epicardial borders in base, mid, apical short axis views **(E–G)** and corresponding LV MyoGCS (LV E_{cc}) **(H)**; left atrial endocardial borders in 2-, and 4-chamber **(I,J)** and corresponding LA endoGLS (LA E_{ll}) **(K)**; right ventricular endocardial border in 4-chamber **(L)** and corresponding RV endoGLS (RV E_{ll}) **(M)**.

TABLE 1 | Summary of clinical characteristics and global left atrium and left ventricle measurements by age category.

Variable	All (*n* = 150)	Men				Women			
		Age				Age			
		≤44	45–59	≥ 60	All (*n* = 75)	≤44	45–59	≥60	All (*n* = 75)
Age, years	51 ± 15	33 ± 7	50 ± 4	66 ± 4	50 ± 15	36 ± 5	53 ± 5	68 ± 6	52 ± 14
Weight, kg	65.4 ± 11.2	72.7 ± 10.6	74.7 ± 10.0	64.2 ± 10.7	70.5 ± 11.2	62.9 ± 9.9	61.4 ± 7.3	59.5 ± 6.9	60.3 ± 8.5
Height, cm	165 ± 8	174 ± 5	172 ± 5	165 ± 7	170 ± 7	163 ± 5	159 ± 5	156 ± 5	159 ± 6
Body mass index, kg/m^2	24.0 ± 2.9	23.9 ± 3.0	25.3 ± 2.8	23.4 ± 3.1	24.2 ± 3	23.6 ± 3.3	24.3 ± 2.4	23.3 ± 2.2	23.7 ± 2.7
Heart rate, bpm	72 ± 14	75 ± 19	70 ± 9	75 ± 16	74 ± 15	72 ± 13	68 ± 9	74 ± 13	71 ± 12
Body surface area, m^2	1.69 ± 0.18	1.84 ± 0.15	1.85 ± 0.15	1.68 ± 0.16	1.79 ± 0.2	1.64 ± 0.14	1.61 ± 0.11	1.52 ± 0.11	1.59 ± 0.1
LV mass, g	74.6 ± 19.9	86.0 ± 18.6	89.4 ± 16.7	83.8 ± 14.7	86.4 ± 16.7	63.9 ± 18.0	63.0 ± 17.2	61.5 ± 10.2	62.8 ± 15.4
LV EDV, mL	114.2 ± 24.2	131.5 ± 27.0	124.6 ± 19.3	119.6 ± 16.0	125.3 ± 21.6	113.3 ± 24.6	103.5 ± 18.5	92.6 ± 15.8	103.1 ± 21.5
LV ESV, mL	45.8 ± 13.6	54.3 ± 15.1	50.8 ± 10.7	45.5 ± 10.0	50.2 ± 12.5	50.0 ± 14.2	40.7 ± 7.8	33.6 ± 12.0	41.5 ± 13.3
LV SV, mL	68.4 ± 14.8	77.2 ± 15.8	73.9 ± 12.8	74.1 ± 11.7	75 ± 13.5	63.2 ± 13.4	62.8 ± 15.0	59.2 ± 10.6	61.8 ± 13.1
LV EF, %	65 ± 7	65 ± 5	65 ± 7	62 ± 6	65 ± 6	64 ± 5	67 ± 6	65 ± 9	65 ± 7
LV mass index, g/m^2	44 ± 9	47 ± 9	48 ± 8	50 ± 7	48 ± 8	38 ± 8	39 ± 10.	41 ± 7	40 ± 9
LV EDV index, mL/m^2	67 ± 11	71 ± 12	67 ± 10	70 ± 9	70 ± 10	69 ± 12	65 ± 10	59 ± 8	64 ± 11
LV ESV index, mL/m^2	27 ± 7	29 ± 8	27 ± 6	27 ± 6	28 ± 7	30 ± 7	25 ± 4	21 ± 7	26 ± 7
LV SV index, mL/m^2	40 ± 7	42 ± 7	40 ± 6	43 ± 6	42 ± 7	38 ± 7	39 ± 9	38 ± 6	38 ± 7
Min. LA volume, mL	26.9 ± 9.8	22.1 ± 6.7	29.1 ± 9.1	31.0 ± 9.8	27.4 ± 9.3	22.3 ± 8.5	24.4 ± 7.9	32.4 ± 11.3	26.4 ± 10.2
Max. LA volume, mL	58.6 ± 18.0	52.9 ± 17.1	62.7 ± 17.7	65.2 ± 17.5	60.3 ± 18.0	54.2 ± 17.5	52.4 ± 16.5	64.4 ± 18.5	57.0 ± 18.1
LAEF, %	58 ± 9	61 ± 7	58 ± 7	54 ± 7	58 ± 8	63 ± 7	62 ± 8	52 ± 10	59 ± 10
Min. LA volume index, mL/m^2	16.0 ± 6.0	11.9 ± 3.1	15.8 ± 4.9	18.4 ± 5.3	15.4 ± 5.2	13.5 ± 5.0	15.2 ± 4.8	21.4 ± 7.3	16.7 ± 6.6
Max. LA volume index, mL/m^2	34.8 ± 10.6	28.7 ± 9.0	34.0 ± 9.6	38.8 ± 9.4	33.9 ± 10.1	32.8 ± 10.2	32.6 ± 9.9	42.2 ± 11.0	35.9 ± 11.2

Data are expressed as mean ± SD or as number (percentage). LV, left ventricular; EDV, end-diastolic volume; ESV, end-systolic volume; SV, stroke volume; EF, ejection fraction; LA, left atrial; Min. LA volume, minimal left atrial volume; Max. LA volume, maximal left atrial volume. The LA EDV, LA ESV and LA EF were from Medis software. Min. LA volume and Max. LA volume were volumes using biplane area-length method.

TABLE 2A | Age and gender global strains of LA endocardium, LV myocardium and RV endocardium.

		All (*n* = 150)	Age				Gender		
			≤44 (*n* = 50)	45–59 (*n* = 50)	≥60 (*n* = 50)	*P*	Male (*n* = 75)	Female (*n* = 75)	*P*
LA	E_{ll}, %	32.8 ± 9.2	36.0 ± 8.7	33.9 ± 8.7	28.7 ± 8.9*#	< 0.001	32.3 ± 9.5	33.3 ± 9.0	0.505
	E_{cc}, %	40.3 ± 13.4	45.0 ± 14.7	42.8 ± 12.2	33.1 ± 10.1*#	< 0.001	39.5 ± 12.1	41.1 ± 14.7	0.473
LV	E_{ll}, %	−22.4 ± 2.9	−23.0 ± 2.7	−22.4 ± 3.1	−20.9 ± 2.7	0.155	−21.6 ± 2.5	−23.3 ± 2.9	< 0.001
	E_{cc}, %	−24.3 ± 3.1	−25.0 ± 2.8	−24.2 ± 3.2	−23.6 ± 3.2	0.060	−23.7 ± 3.1	−24.9 ± 3.1	0.016
	E_{rr}, %	79.0 ± 19.4	72.9 ± 15.9	78.1 ± 20.8*	86.3 ± 19.2*#	0.002	76.2 ± 17.9	81.9 ± 20.6	0.075
RV	E_{ll}, %	−29.3 ± 6.0	−30.0 ± 6.1	−29.1 ± 6.7	−28.8 ± 5.0	0.559	−28.5 ± 6.4	−30.1 ± 5.5	0.104

*Data are expressed as mean ± SD. LA, left atrial; LV, left ventricle; RV, right ventricle; E_{ll}, global longitudinal strain; E_{cc}, global circumferential strain; E_{rr}, global radial strain. *Difference between the young and the old was significant ($P < 0.05$). #Difference between the middle aged and the old was significant ($P < 0.05$).*

TABLE 2B | Age and gender global strains of LA endocardium, LV myocardium and RV endocardium.

		≤44			45–59			≥60		
		Male (*n* = 25)	Female (*n* = 25)	*P*	Male (*n* = 25)	Female (*n* = 25)	*P*	Male (*n* = 25)	Female (*n* = 25)	*P*
LA	E_{ll}, %	35.5 ± 9.6	36.4 ± 7.8	0.738	31.8 ± 8.5	35.9 ± 8.5	0.096	29.6 ± 9.7	27.7 ± 8.0	0.454
	E_{cc}, %	44.0 ± 15.3	45.9 ± 14.4	0.651	40.7 ± 9.8	44.8 ± 14.1	0.235	33.7 ± 7.9	32.5 ± 12.0	0.647
LV	E_{ll}, %	−22.4 ± 2.0	−23.6 ± 3.1	0.119	−21.4 ± 3	−23.3 ± 2.8	0.021*	−20.9 ± 2.2	−22.9 ± 2.9	0.009#
	E_{cc}, %	−25.4 ± 2.9	−24.7 ± 2.8	0.415	−23.3 ± 3.1	−25.1 ± 3	0.040*	−22.3 ± 2.4	−24.8 ± 3.5	0.005#
	E_{rr}, %	73.7 ± 13.9	71.9 ± 18.0	0.687	72.5 ± 19.5	83.6 ± 20.8	0.059	82.4 ± 18.6	90.2 ± 19.4	0.156
RV	E_{ll}, %	−30.6 ± 6.1	−29.4 ± 6.2	0.482	−24.5 ± 14.2	−28.3 ± 13.7	0.334	−27.8 ± 4.0	−29.8 ± 5.8	0.164

**Difference between the middle-aged men and women was significant ($P < 0.05$). #Difference between the old men and women was significant ($P < 0.05$).*

and one in Singapore: (1) no symptoms or prior history of cardiovascular or cerebrovascular disease; (2) no prior diagnosis of hyperlipidaemia, hypertension or diabetes mellitus; (3) normal physical examination and electrocardiogram; (4) no contraindications to CMR. Subjects with wall motion abnormalities or significant valvular diseases detected on CMR were excluded. The study was approved by the local institutional research ethics committee. Written informed consent was obtained from all subjects. The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in *a priori* approval by the institution's human research committee.

CMR Image Acquisition

Subjects underwent cine CMR on a 1.5T (Verio, Siemens, Germany) or a 3.0T (Prisma, Siemens, Germany) CMR scanners at the China sites and on a 3.0T (3.0T, Ingenia, Philips Healthcare, Netherlands) CMR scanner at the Singapore site, using a standardized imaging protocol (Kramer et al., 2013). Balanced SSFP (China sites) and BTFE (Singapore site) sequences with breath-hold were performed to obtain cine CMR images, comprising a stack of contiguous parallel short-axis slices covering the entire LV and RV from base to apex and three LV long-axis slice (2-, 3-, and 4-chamber views) images. The slice thickness/spacing is 5 mm/1 mm for long axis and 8 mm/2 mm for short axis in China sites; and 8 mm/0 mm for both short and long axis in Singapore site.

CMR Image Post-processing

Cine CMR image manual segmentation and analyses were conducted by investigators experienced in CMR (JPP, XDZ, and ZW). Minimal and maximal left atrial volumes were calculated at the respective cardiac phases using biplane area-length method (Lang et al., 2005): LA volume (mL) = 0.85*A2C*A4C/L, where A2C and A4C represent planimetered LA area in the 2- and 4-chamber views, respectively; and L, the length of the major LA axis at either the 2- or 4-chamber view, whichever is shorter. LV end-diastolic volume (LVEDV), end-systolic volume (LVESV), stroke volume (LVSV), ejection fraction (LVEF) and LV mass were measured using Qmass (Medis Suite, Netherlands), and applicable values indexed to body surface area were calculated.

CMR Feature Tracking Analysis

Global and regional LA, LV, and RV strain and systolic strain rate measurements were analyzed using commercial cardiovascular post-processing software (Medis 3.0, Netherlands). Feature tracking allows quantification of global and regional longitudinal, circumferential and radial strain and strain rates. A short description of the analysis is given here. At end-diastole, endocardial and epicardial borders were manually delineated using a point-and-click approach before the automated tracking algorithm was applied. Papillary muscles included within the endocardial borders (**Figures 1A–C,E–G**). LA endocardial contours were initially traced in the 2- and 4- chamber views at the minimum LA volume after atrial contraction (**Figures 1I,J**). RV endocardial border was traced in 4-chamber view (**Figure 1L**). Guided by signal in homogeneities or anatomical features and using a maximum likelihood method, the software algorithm provides an automatically traced image with frame-to-frame template matching throughout the entire cardiac cycle. Strain values are derived by comparing the movement of the features in relation to each other along the initially drawn borders. CMR feature tracking performance was visually reviewed to ensure accurate tracking. In cases where tracking is determined to be inadequate, the software allows for border editing. Global longitudinal, circumferential and radial strain values (E_{ll}, E_{cc}, and E_{rr}) were automatically extracted from corresponding strain curves (**Figures 1D,H,K,M**).

LA longitudinal strain and strain rate were measured in both 2- and 4-chamber views, excluding the confluence of the pulmonary veins and LA appendage in the border delineation. The LA wall contours were automatically segmented into anterior, inferior, posterior, lateral and atrial septal regions by the software (Kowallick et al., 2014; Zareian et al., 2015).

The standard 17-segment model of the American Heart Association (AHA) (Cerqueira et al., 2002) was applied in the analysis of LV longitudinal strain measured in 2-, 3-, and 4-chamber views. The basal, mid-cavity and apical levels were segmented from the end-diastolic 4-chamber long-axis cine image. For circumferential and radial strain analyses of LV short-axis views, a modified 16-segment model (omitting the apical cap) was applied, using the RV insertion point as the reference point for the junction of LV anterior wall and septum. For short axis strain analysis, base was selected as the slice still showing a complete circumference of myocardium throughout the entire cardiac cycle (without through plane distortion from the LV outflow tract) and the apex was selected as the slice still showing LV cavity at systole (Taylor et al., 2015; Kowallick et al., 2016).

Segmental endocardial and myocardial values of LV were separately measured for longitudinal and circumferential strain and strain rates. To dissect the regional strain distribution, we calculated longitudinal and circumferential strains at basal, middle and apical levels, as well as at the anterior wall, septal, inferior wall and lateral walls of LV by averaging the peak values of the segments corresponding to the relevant territories. For each segment, strain rate was calculated as the derivative of the initial measured strain.

Global LA longitudinal strain (and strain rate) was calculated as the average of strain (and strain rate) from 2-and 4-chamber views, and regional segments were divided as anterior wall, inferior wall, roof, lateral wall and septum wall. The values at roof was taken as the average value from 2- and 4-chamber views. Global and regional RV (free wall and septum) longitudinal strains were measured on the 4-chamber view, and the respective strain rates were calculated (Heermann et al., 2014; Prati et al., 2015).

Statistical Analysis

All continuous variables were described as mean ± standard deviation (SD). Means of baseline variables among the three age groups were compared using one-way analysis of variance (ANOVA) with *post hoc* pairwise comparisons and the Bonferroni correction for multiple comparisons;

TABLE 3 | Left ventricular segmental endocardial and myocardial circumferential strain and strain rates from short axis view.

Segment	Circumferential endocardial strain, %	Circumferential endocardial strain rate, s^{-1}	Circumferential myocardial strain, %	Circumferential myocardial strain rate, s^{-1}
1	−33.2 ± 8.9	−1.80 ± 0.55	−21.1 ± 6.8	−1.12 ± 0.37
2	−33.9 ± 8.5	−1.87 ± 0.61	−20.7 ± 6.4	−1.07 ± 0.37
3	−31.1 ± 8.4	−1.63 ± 0.55	−23.6 ± 6.1	−1.16 ± 0.38
4	−31.4 ± 7.6	−1.58 ± 0.49	−20.8 ± 6.5	−0.94 ± 0.33
5	−37.3 ± 6.3	−2.07 ± 0.55	−28.8 ± 6.5	−1.46 ± 0.41
6	−38.6 ± 7.2	−2.08 ± 0.58	−31.3 ± 7.3	−1.61 ± 0.50
7	−34.0 ± 8.6	−1.92 ± 0.63	−18.4 ± 6.6	−1.01 ± 0.35
8	−34.6 ± 9.5	−1.96 ± 0.71	−19.7 ± 6.5	−1.07 ± 0.43
9	−32.3 ± 8.6	−1.75 ± 0.58	−23.7 ± 5.4	−1.18 ± 0.33
10	−31.8 ± 7.2	−1.66 ± 0.46	−21.5 ± 5.8	−1.07 ± 0.32
11	−36.0 ± 6.4	−1.98 ± 0.57	−25.5 ± 6.2	−1.36 ± 0.46
12	−29.6 ± 9.0	−1.60 ± 0.58	−22.6 ± 9.1	−1.25 ± 0.43
13	−41.3 ± 9.2	−2.34 ± 0.79	−21.8 ± 8.0	−1.19 ± 0.48
14	−44.1 ± 9.6	−2.48 ± 0.84	−27.6 ± 7.1	−1.44 ± 0.44
15	−45.3 ± 9.9	−2.53 ± 0.86	−32.1 ± 7.2	−1.65 ± 0.53
16	−42.5 ± 9.6	−2.45 ± 0.85	−29.1 ± 7.6	−1.63 ± 0.52

Data are expressed as mean ± SD. 1, basal anterior; 2, basal anterolateral; 3, basal inferoseptal; 4, basal inferior; 5, basal inferolateral; 6, basal anterolateral; 7, mid anterior; 8, mid anteroseptal; 9, mid inferoseptal; 10, mid inferior; 11, mid inferolateral; 12, mid anterolateral; 13, apical anterior; 14, apical septal; 15, apical inferior; 16, apical lateral.

comparisons between males and females were performed using a two-sample *t*-test. Correlation was assessed using the Pearson correlation coefficient (*r*). Statistical significance was set at $P \leq 0.05$. All analyses were performed using SPSS Statistics 22.0. Intra- and inter-observer reproducibility were performed in 20 randomly selected subjects using intra-class coefficient (ICC), Bland–Altman method and the coefficient of variation (CV), which was calculated as the mean of absolute difference between two methods over the mean average.

RESULTS

Clinical Characteristics of Study Subjects

One hundred and fifty subjects (75 Males/75 Females, 18–82 years) with cine CMR images acceptable for CMR feature tracking analysis were recruited. A summary of clinical characteristics and global left atrium and left ventricle measurements by age category are presented in **Table 1**. Of the 150 subjects, 84 were from Beijing Anzhen Hospital [40 males, mean (±SD) age 43 ± 12 years], 41 from National Heart Centre Singapore (24 males, mean age 66 ± 8 years), and 25 from Longgang Central Hospital of Shenzhen (12 males; mean age 54 ± 11 years).

Global and Regional Strain Values

Normal values for global LA endocardial, LV myocardial and RV endocardial CMR feature-tracking deformation measured parameters are shown in **Table 2A**. Mean endocardial LA E_{ll} and LA E_{cc} were 32.8 ± 9.2 and 40.3 ± 13.4%, respectively. For LA E_{ll} at the segmental level, the LA roof exhibited the lowest strain (30.7 ± 13.9%), and differences between the LA roof and LA E_{ll} at the anterior (38.7 ± 18.5%) and inferior (41.3 ± 20.4%) walls were both significant ($P \leq 0.003$). LA strain rate at the anterior wall (1.66 ± 0.89 s^{-1}) was significantly higher than at the LA roof (1.35 ± 0.52 s^{-1}), lateral wall (1.30 ± 0.63 s^{-1}) and septal wall (1.40 ± 0.61 s^{-1}) (all $P \leq 0.01$). Age and gender specific global LA endocardial, LV myocardial and RV endocardial strain values are given in **Table 2B**.

For LV myocardium strains, mean LV E_{ll}, LV E_{cc} and LV E_{rr} was −22.4 ± 2.9, −24.3 ± 3.1, and 79.0 ± 19.4%, respectively. The endocardial and myocardial circumferential strain and strain rates for regional 16 segments are provided in **Table 3**. Barchart plots (with one standard deviation) for segmental LV endocardial and myocardial circumferential strains and strain rates at basal, mid-cavity and apical levels, and at the anterior, septum, inferior and lateral walls are given in **Figures 2A–D**. Means for both endocardial and myocardial LV E_{cc} were significantly higher at the apical level than at the base and mid-cavity levels (all $P < 0.001$), and the mid-level exhibited the lowest strain among the three levels ($P < 0.001$). For both endocardial and myocardial circumferential strain rates, significantly larger values were observed at the apical level compared to mid-cavity ($P < 0.001$). Furthermore, myocardial E_{cc} and strain rate increased significantly from anterior → septum → inferior → lateral walls (all $P < 0.001$), while no differences were found for endocardial E_{cc} and strain rate among these four regional walls. In contrast, endocardial LV E_{ll} increased significantly from basal to mid to apex (**Figure 2E**), and mean myocardial LV E_{ll} was higher at the middle level rather than at the basal and apical levels (**Figure 2G**). Lateral walls had significantly higher endocardial and myocardial E_{ll} and strain rates than anterior walls, and inferior and septum walls, with septum walls having the lowest strain and strain rates

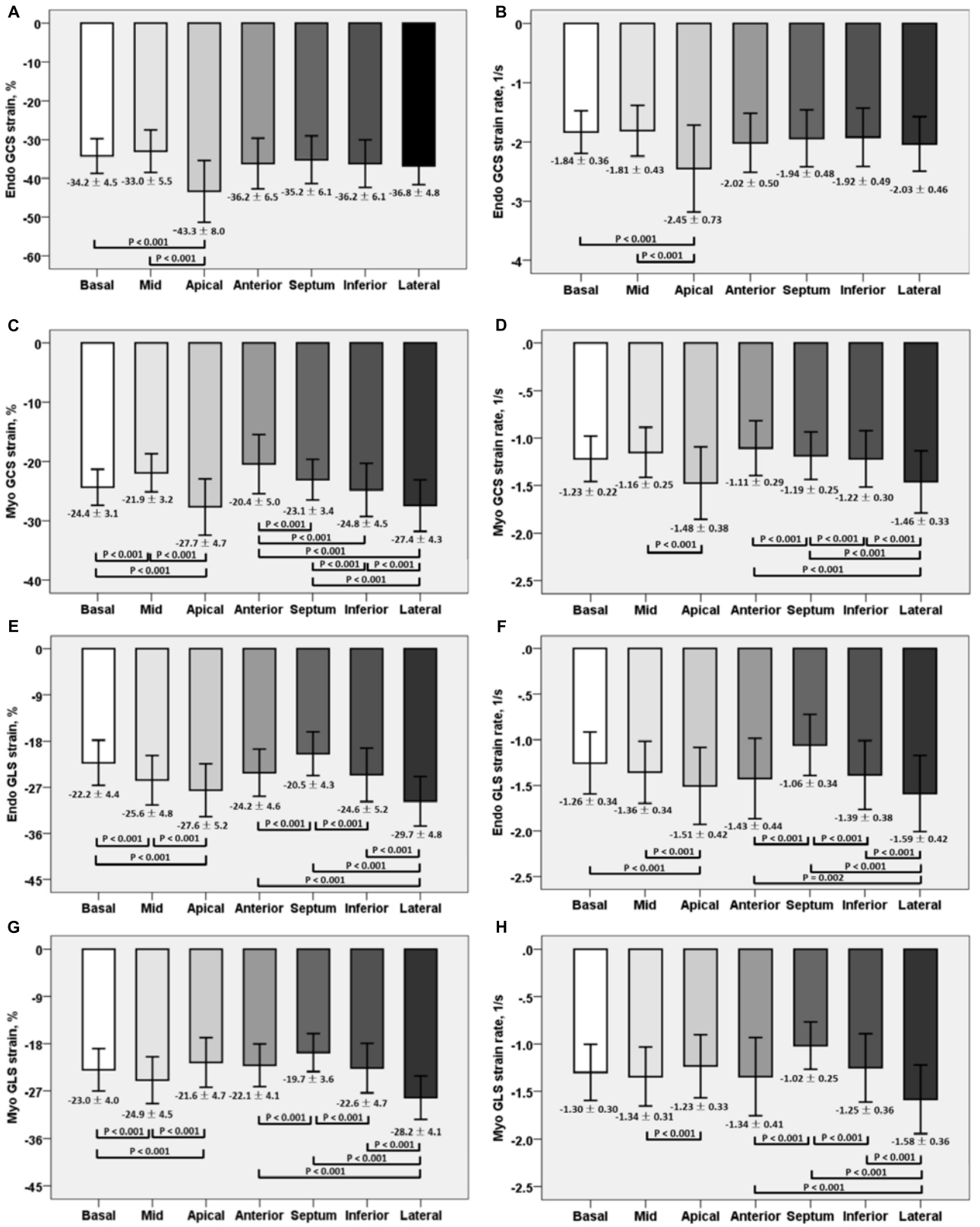

FIGURE 2 | Comparison of endocardial circumferential strain and strain rate **(A)** and **(B)**; myocardial circumferential strain and strain rate **(C)** and **(D)**; endocardial longitudinal strain and strain rate **(E)** and **(F)**; and myocardial longitudinal strain and strain rate **(G)** and **(H)** at basal, mid and apical levels; anterior, septum, inferior and lateral walls.

TABLE 4 | Left ventricular segmental endocardial and myocardial longitudinal strain and strain rates from long axis view.

Segment	Longitudinal endocardial strain, %	Longitudinal endocardial strain rate, s^{-1}	Longitudinal myocardial strain, %	Longitudinal myocardial strain rate, s^{-1}
1	−14.8 ± 7.8	−1.01 ± 0.58	−12.6 ± 7.5	−0.98 ± 0.59
2	−15.3 ± 9.1	−0.93 ± 0.46	−19.0 ± 8.1	−1.03 ± 0.44
3	−18.8 ± 6.9	−0.88 ± 0.55	−18.1 ± 6.3	−0.88 ± 0.32
4	−20.1 ± 8.8	−1.29 ± 0.60	−22.1 ± 8.8	−1.30 ± 0.60
5	−32.5 ± 8.0	−1.79 ± 0.53	−33.4 ± 8.8	−1.84 ± 0.58
6	−31.7 ± 8.6	−1.64 ± 0.68	−32.9 ± 8.8	−1.77 ± 0.59
7	−23.9 ± 10.8	−1.41 ± 0.63	−23.1 ± 10.8	−1.35 ± 0.61
8	−22.9 ± 7.9	−1.22 ± 0.47	−20.7 ± 7.8	−1.10 ± 0.44
9	−23.7 ± 8.0	−1.11 ± 0.59	−24.0 ± 7.4	−1.17 ± 0.44
10	−22.5 ± 9.8	−1.20 ± 0.49	−23.5 ± 9.4	−1.23 ± 0.51
11	−31.0 ± 8.8	−1.62 ± 0.60	−29.7 ± 10.4	−1.62 ± 0.65
12	−29.8 ± 9.8	−1.57 ± 0.73	−28.5 ± 10.5	−1.59 ± 0.67
13	−33.9 ± 8.9	−1.86 ± 0.69	−30.7 ± 9.9	−1.70 ± 0.66
14	−21.7 ± 6.7	−1.16 ± 0.42	−16.6 ± 5.7	−0.91 ± 0.32
15	−31.1 ± 9.6	−1.67 ± 0.64	−22.3 ± 10.7	−1.22 ± 0.53
16	−23.6 ± 8.5	−1.33 ± 0.52	−16.6 ± 7.9	−1.10 ± 0.43
17	−31.6 ± 5.9	−1.69 ± 0.50	−23.9 ± 5.6	−1.26 ± 0.40

Data are expressed as mean ± SD. 1, basal anterior; 2, basal anterolateral; 3, basal inferoseptal; 4, basal inferior; 5, basal inferolateral; 6, basal anterolateral; 7, mid anterior; 8, mid anteroseptal; 9, mid inferoseptal; 10, mid inferior; 11, mid inferolateral; 12, mid anterolateral; 13, apical anterior; 14, apical septal; 15, apical inferior; 16, apical lateral; 17, apex.

(**Figures 2F,H**). Endocardial and myocardial longitudinal strain and strain rates for the regional 17 segments are provided in **Table 4**.

Mean RV E_{ll} was −29.3 ± 6.0%, with RV free wall exhibiting greater longitudinal strain (−35.3 ± 7.4% vs. −23.9 ± 6.9%) and strain rate (−1.83 ± 0.46 s^{-1} vs. −1.12 ± 0.39 s^{-1}) than the septum (both $P < 0.001$).

Age and Gender Difference in Global and Regional Strain and Strain Rate

Global strain values for LA, LV, and RV among age groups is given in **Table 2**. For age groups 1, 2, and 3, respectively, global strain values were 36.0 ± 8.7, 33.9 ± 8.7, and 28.7 ± 8.9% for LA E_{ll}, and 45.0 ± 14.7, 42.8 ± 12.2, and 33.1 ± 10.1% for LA E_{cc}. LA E_{ll} and E_{cc} decreased in magnitude with increasing age; correlations were statistically significant but weak ($r = -0.330$ for LA E_{ll} and $r = -0.364$ for LA E_{cc}). LA endocardial strain differences between male and female were non-significant, with values of 32.3 ± 9.5% vs. 33.3 ± 8.9% for E_{ll} ($P = 0.505$) and 39.5 ± 12.1% vs. 41.1 ± 14.7% for E_{cc} ($P = 0.473$).

In age groups 1, 2, and 3, mean values for LV myocardial E_{ll} were −23.0 ± 2.7, −22.4 ± 3.1, and −20.9 ± 2.7; for LV E_{cc} −25.0 ± 2.8, −24.2 ± 3.2, and −23.6 ± 3.2%; and for LV E_{rr} 72.8 ± 15.9, 78.1 ± 20.8, and 86.3 ± 19.2%. Linear regression showed weak but statistically significant associations, respectively, of E_{cc} and E_{rr} with age ($r = 0.208$ and $r = 0.258$, both $P < 0.05$). Differences between males and females were significant for LV E_{ll} (−21.6 ± 2.5% vs. −23.3 ± 2.9%) and LV E_{cc} (−23.7 ± 3.1% vs. −24.9 ± 3.1%) (both $P < 0.05$). Difference in LV E_{rr} between genders (76.2 ± 17.9% vs. 81.9 ± 20.6%) was non-significant ($P = 0.075$). Comparison of male and female for three age groups with P-value was tabulated in **Table 2B**. Age and gender specific endocardial and myocardial E_{cc} values for 16 segments are given in **Tables 5**, **6**, and endocardial and myocardial E_{ll} for 17 segments are tabulated in **Tables 7**, **8**.

RV E_{ll} showed no significant differences among three age groups (−30.0 ± 6.1, −29.1 ± 6.7, and −28.8 ± 5.0%, $P = 0.559$). E_{ll} values in males and females were −28.5 ± 6.4 and −30.1 ± 5.5%, but not statistically significant ($P = 0.104$).

Reproducibility

The intra- and inter-observer variability results were given in **Table 9**. In Bland–Altman analyses, LV E_{cc} had the best intra-observer agreement (bias, −0.04 ± 0.72; 95% CI, −1.48 to 1.41), and LV E_{ll} had the best inter-observer agreement (bias, −0.07 ± 0.77; 95% CI, −1.61 to 1.47). All parameters had an intra- and inter- observer ICC > 0.89, except for LV E_{rr} with intra- (0.793) and inter- (0.832) observer.

DISCUSSION

To the best of our knowledge, this is the largest prospective study to date that quantifies global, segmental and regional strain and strain rates of healthy Chinese across a broad age range. Based on results obtained using the CMR feature tracking technique, our study demonstrates (i) higher magnitudes of longitudinal and circumferential LA and LV strain in females; (ii) regional variations in longitudinal and circumferential strains, with higher strain values in the lateral LV territories and RV free wall compared to the septal area; and (iii) independent associations between age and LA and LV global circumferential

TABLE 5 | Left ventricular circumferential endocardial peak systolic strain values from short axis view.

Segment	Men				Women			
	Age				Age			
	≤44	45–59	≥60	All	≤44	45–59	≥60	All
1	−31.1 ± 6.9	−30.9 ± 6.8	−34.5 ± 6.0	−32.2 ± 6.7	−30.5 ± 8.6	−35.8 ± 11.1	−36.3 ± 11.2	−34.2 ± 10.6
2	−34.7 ± 9.3	−31.7 ± 7.5	−32.2 ± 9.5	−32.9 ± 8.8	−34.2 ± 6.9	−35.4 ± 6.9	−35.2 ± 10.4	−34.9 ± 8.1
3	−26.6 ± 8.4	−31.1 ± 6.2	−32.5 ± 7.1	−30.1 ± 7.6	−28.5 ± 8.2	−29.6 ± 7.0	−38.2 ± 8.9	−32.1 ± 9.1
4	−30.1 ± 5.6	−32.6 ± 6.5	−27.7 ± 8.6	−30.2 ± 7.2	−31.3 ± 7.4	−33.1 ± 8.3	−33.3 ± 8.3	−32.6 ± 7.9
5	−36.9 ± 5.2	−36.0 ± 5.6	−34.5 ± 5.5	−35.8 ± 5.4	−38.2 ± 6.0	−40.0 ± 5.9	−38.7 ± 8.4	−38.8 ± 6.8
6	−38.1 ± 6.2	−34.9 ± 7.8	−36.3 ± 5.7	−36.4 ± 6.7	−41.3 ± 6.5	−40.8 ± 5.2	−40.5 ± 8.9	−40.9 ± 7.0
7	−36.9 ± 5.9	−33.3 ± 8.9	−34.5 ± 6.0	−34.2 ± 7.5	−32.3 ± 9.6	−31.7 ± 8.3	−37.4 ± 10.3	−33.8 ± 9.7
8	−33.2 ± 7.6	−34.8 ± 9.2	−33.8 ± 9.4	−33.9 ± 8.7	−30.2 ± 9.8	−36.2 ± 10.8	−39.4 ± 8.0	−35.2 ± 10.2
9	−29.7 ± 7.9	−33.1 ± 8.1	−34.6 ± 8.6	−32.5 ± 8.4	−27.5 ± 8.8	−32.8 ± 7.0	−35.9 ± 8.9	−32.1 ± 8.9
10	−29.3 ± 6.5	−32.4 ± 7.9	−30.1 ± 7.6	−30.6 ± 7.4	−31.9 ± 7.6	−32.1 ± 6.9	−35.0 ± 6.2	−33.0 ± 7.0
11	−37.6 ± 4.9	−35.6 ± 6.5	−33.0 ± 6.5	−35.4 ± 6.3	−35.2 ± 6.0	−37.8 ± 5.8	−36.5 ± 7.7	−36.5 ± 6.5
12	−30.9 ± 6.4	−30.7 ± 10.3	−27.1 ± 8.8	−29.6 ± 8.7	−28.8 ± 8.4	−29.8 ± 9.8	−30.8 ± 10.1	−29.7 ± 9.4
13	−44.5 ± 10.8	−41.3 ± 10.3	−38.5 ± 7.1	−38.5 ± 7.1	−40.7 ± 9.6	−39.3 ± 8.0	−43.5 ± 8.1	−41.1 ± 8.7
14	−43.8 ± 10.1	−45.0 ± 8.9	−43.5 ± 6.9	−44.1 ± 8.7	−40.3 ± 12.3	−45.6 ± 10.1	−46.6 ± 7.7	−44.2 ± 10.4
15	−49.5 ± 10.8	−46.1 ± 9.0	−40.6 ± 9.8	−45.4 ± 10.5	−43.7 ± 11.2	−46.0 ± 9.0	−46.2 ± 8.2	−45.3 ± 9.5
16	−49.2 ± 9.6	−42.7 ± 9.6	−38.3 ± 8.6	−43.4 ± 10.2	−42.1 ± 9.4	−42.0 ± 7.5	−40.8 ± 10.1	−41.6 ± 9.0
Basal	−32.9 ± 3.6	−32.9 ± 3.4	−33.0 ± 3.9	−32.9 ± 3.6	−34.0 ± 4.6	−35.7 ± 4.1	−37.0 ± 5.7	−35.6 ± 5.0
Mid	−32.9 ± 4.0	−33.3 ± 6.4	−31.8 ± 4.8	−32.7 ± 5.1	−31.0 ± 5.4	−33.4 ± 5.6	−35.8 ± 5.7	−33.4 ± 5.8
Apical	−46.7 ± 9.0	−43.8 ± 8.5	−40.2 ± 8.6	−43.6 ± 8.4	−41.7 ± 8.7	−43.2 ± 7.0	−44.2 ± 6.7	−43.0 ± 7.5

Data are expressed as mean ± SD.

TABLE 6 | Left ventricular circumferential myocardial peak systolic strain values from short axis view.

Segment	Men				Women			
	Age				Age			
	≤44	45–59	≥60	All	≤44	45–59	≥60	All
1	−20.3 ± 6.4	−19.0 ± 5.6	−22.0 ± 6.1	−20.5 ± 6.1	−20.7 ± 6.0	−21.3 ± 8.9	−22.9 ± 7.0	−21.6 ± 7.4
2	−21.7 ± 6.0	−17.6 ± 6.1	−20.2 ± 7.2	−19.8 ± 6.6	−21.3 ± 5.1	−20.9 ± 6.2	−22.6 ± 6.7	−21.6 ± 6.0
3	−20.7 ± 6.3	−22.6 ± 5.2	−24.0 ± 6.1	−22.4 ± 6.0	−23.8 ± 7.0	−23.8 ± 5.2	−27.0 ± 5.3	−24.9 ± 6.0
4	−19.5 ± 5.9	−21.6 ± 7.0	−19.5 ± 5.0	−20.2 ± 6.0	−21.9 ± 6.6	−22.3 ± 5.8	−19.8 ± 8.3	−21.3 ± 7.0
5	−28.2 ± 4.7	−26.6 ± 5.9	−27.3 ± 6.1	−27.4 ± 5.6	−30.7 ± 5.1	−30.4 ± 6.0	−29.5 ± 9.3	−30.2 ± 7.0
6	−31.9 ± 6.4	−27.4 ± 8.2	−28.7 ± 4.7	−29.3 ± 6.8	−35.0 ± 7.5	−32.7 ± 7.1	−31.8 ± 7.3	−33.2 ± 7.3
7	−19.4 ± 6.8	−17.3 ± 5.5	−19.7 ± 5.9	−18.8 ± 6.1	−18.1 ± 7.0	−15.0 ± 6.1	−20.9 ± 7.1	−18.0 ± 7.1
8	−19.2 ± 5.7	−19.1 ± 7.2	−18.2 ± 5.3	−18.9 ± 6.0	−18.8 ± 6.4	−19.9 ± 7.5	−22.7 ± 6.2	−20.5 ± 6.9
9	−23.8 ± 3.5	−23.8 ± 5.1	−23.2 ± 5.4	−23.6 ± 4.7	−22.3 ± 5.6	−25.0 ± 5.8	−24.0 ± 6.5	−23.8 ± 6.0
10	−18.9 ± 5.8	−21.8 ± 5.0	−22.3 ± 6.3	−21.0 ± 5.8	−21.3 ± 5.4	−21.2 ± 5.6	−23.6 ± 6.3	−22.0 ± 5.8
11	−27.7 ± 4.9	−24.0 ± 6.6	−22.1 ± 6.3	−24.6 ± 6.3	−27.0 ± 5.6	−27.5 ± 4.6	−24.5 ± 7.0	−26.3 ± 5.9
12	−23.4 ± 7.0	−21.9 ± 9.6	−19.4 ± 9.1	−21.6 ± 8.7	−21.7 ± 8.9	−24.5 ± 11.4	−24.7 ± 7.9	−23.6 ± 9.5
13	−25.2 ± 10.2	−21.3 ± 7.4	−20.6 ± 5.9	−22.3 ± 8.2	−22.1 ± 8.4	−18.6 ± 8.2	−23.3 ± 5.9	−21.3 ± 7.8
14	−27.5 ± 6.0	−26.0 ± 6.3	−27.3 ± 4.5	−26.9 ± 5.6	−26.4 ± 8.0	−30.3 ± 7.4	−28.2 ± 9.5	−28.3 ± 8.4
15	−34.9 ± 7.2	−32.5 ± 5.5	−29.1 ± 7.4	−32.1 ± 7.1	−32.0 ± 8.5	−32.4 ± 7.4	−31.8 ± 6.5	−32.1 ± 7.4
16	−34.7 ± 5.8	−28.8 ± 7.1	−23.2 ± 5.7	−28.9 ± 7.8	−30.8 ± 6.7	−31.1 ± 7.4	−26.2 ± 7.8	−29.4 ± 7.6
Basal	−23.7 ± 2.7	−22.5 ± 2.3	−23.6 ± 2.5	−23.3 ± 2.5	−25.5 ± 3.0	−25.0 ± 2.8	−25.6 ± 3.7	−25.4 ± 3.2
Mid	−22.1 ± 2.4	−21.3 ± 3.2	−20.8 ± 3.3	−21.4 ± 3.0	−21.6 ± 2.9	−22.2 ± 3.6	−23.4 ± 3.6	−22.4 ± 3.4
Apical	−30.6 ± 5.2	−27.1 ± 4.8	−25.0 ± 3.4	−27.6 ± 5.0	−27.8 ± 4.6	−28.1 ± 4.7	−27.4 ± 4.2	−27.8 ± 4.5

Data are expressed as mean ± SD.

TABLE 7 | Left ventricular longitudinal endocardial peak systolic strain values from long axis view.

Segment	Men				Women			
	Age				Age			
	≤44	45–59	≥60	All	≤44	45–59	≥60	All
1	−17.4 ± 7.7	−12.9 ± 7.0	−15.6 ± 7.9	−15.3 ± 7.7	−15.6 ± 8.4	−14.6 ± 8.0	−12.6 ± 7.5	−14.2 ± 8.0
2	−18.5 ± 7.6	−13.8 ± 5.6	−9.4 ± 8.2	−13.9 ± 8.0	−20.7 ± 8.9	−18.4 ± 7.2	−11.1 ± 10.8	−16.7 ± 9.9
3	−20.9 ± 5.5	−17.5 ± 5.4	−20.7 ± 6.0	−19.7 ± 5.8	−18.5 ± 4.6	−20.3 ± 8.6	−14.8 ± 8.6	−17.9 ± 7.8
4	−17.2 ± 7.6	−18.9 ± 8.6	−20.8 ± 7.4	−19.0 ± 8.0	−22.3 ± 8.5	−24.8 ± 8.3	−16.6 ± 10.0	−21.3 ± 9.5
5	−33.3 ± 7.6	−30.1 ± 8.6	−30.9 ± 6.7	−31.4 ± 7.7	−32.8 ± 6.5	−35.6 ± 8.7	−32.6 ± 9.3	−33.7 ± 8.3
6	−28.9 ± 7.7	−31.3 ± 7.5	−28.7 ± 8.6	−29.6 ± 7.9	−33.1 ± 7.7	−32.7 ± 10.2	−35.9 ± 8.3	−33.9 ± 8.8
7	−17.7 ± 7.2	−22.1 ± 9.9	−23.8 ± 11.9	−21.2 ± 10.1	−27.2 ± 10.0	−26.2 ± 10.7	−26.2 ± 12.2	−26.6 ± 10.9
8	−22.6 ± 8.8	−21.8 ± 8.3	−21.1 ± 8.1	−21.9 ± 8.3	−25.0 ± 6.9	−25.0 ± 5.0	−22.0 ± 9.7	−24.0 ± 7.5
9	−23.3 ± 7.2	−23.0 ± 9.2	−23.2 ± 6.7	−23.1 ± 7.6	−25.0 ± 6.8	−25.5 ± 8.3	−22.4 ± 9.6	−24.3 ± 8.3
10	−23.6 ± 9.8	−20.5 ± 6.9	−18.8 ± 11.0	−20.9 ± 9.5	−25.9 ± 10.0	−27.8 ± 9.0	−18.5 ± 8.6	−24.1 ± 10.0
11	−28.4 ± 8.6	−31.5 ± 6.7	−26.1 ± 7.8	−28.7 ± 7.9	−33.7 ± 8.3	−34.5 ± 8.4	−31.7 ± 10.2	−33.3 ± 9.0
12	−25.5 ± 11.6	−28.2 ± 9.5	−27.9 ± 8.0	−27.2 ± 9.8	−31.3 ± 7.9	−31.5 ± 11.1	−34.1 ± 8.5	−32.3 ± 9.2
13	−32.9 ± 7.4	−32.0 ± 7.3	−30.2 ± 9.5	−31.7 ± 8.1	−34.5 ± 8.9	−35.9 ± 9.5	−37.6 ± 9.0	−36.0 ± 9.1
14	−23.6 ± 5.5	−21.2 ± 7.9	−20.1 ± 5.0	−21.6 ± 6.4	−21.9 ± 8.2	−19.3 ± 6.7	−24.0 ± 5.6	−21.7 ± 7.1
15	−34.3 ± 8.1	−30.2 ± 10.7	−28.7 ± 9.5	−31.1 ± 9.7	−32.6 ± 9.4	−27.6 ± 10.1	−33.4 ± 8.6	−31.2 ± 9.6
16	−26.2 ± 7.3	−25.8 ± 9.1	−22.4 ± 4.7	−24.8 ± 7.3	−23.7 ± 9.9	−20.7 ± 10.5	−23.0 ± 7.7	−22.5 ± 9.4
17	−35.3 ± 5.3	−31.7 ± 5.4	−29.7 ± 5.6	−32.2 ± 5.9	−30.9 ± 6.6	−29.2 ± 5.8	−32.8 ± 5.2	−31.0 ± 6.0
Basal	−22.7 ± 2.7	−20.7 ± 3.3	−21.0 ± 4.3	−21.5 ± 3.5	−23.9 ± 3.7	−24.4 ± 5.2	−20.6 ± 5.3	−22.9 ± 5.0
Mid	−23.5 ± 3.9	−24.5 ± 4.3	−23.5 ± 4.7	−23.8 ± 4.3	−28.0 ± 3.0	−28.4 ± 4.4	−25.8 ± 5.8	−27.4 ± 4.6
Apical	−29.2 ± 4.2	−27.3 ± 5.5	−25.3 ± 4.5	−27.3 ± 5.0	−28.2 ± 5.9	−25.9 ± 5.7	−29.5 ± 4.1	−27.8 ± 5.4
Anterior	−22.7 ± 3.9	−22.4 ± 4.2	−23.2 ± 4.3	−22.7 ± 4.1	−25.8 ± 4.7	−25.6 ± 5.0	−25.5 ± 4.5	−25.6 ± 4.7
Septum	−21.8 ± 3.2	−19.5 ± 3.6	−18.9 ± 4.0	−20.0 ± 3.8	−22.2 ± 2.7	−21.7 ± 4.3	−18.9 ± 6.0	−20.9 ± 4.7
Inferior	−25.0 ± 5.0	−23.0 ± 4.4	−22.8 ± 5.1	−23.7 ± 4.9	−26.9 ± 6.5	−26.7 ± 3.9	−22.8 ± 4.7	−25.5 ± 5.4
Lateral	−28.5 ± 4.0	−29.4 ± 5.2	−27.2 ± 3.7	−28.3 ± 4.4	−30.9 ± 3.0	−31.0 ± 5.6	−31.4 ± 5.6	−31.1 ± 4.8

Data are expressed as mean ± SD. 1, basal anterior; 2, basal anterolateral; 3, basal inferoseptal; 4, basal inferior; 5, basal inferolateral; 6, basal anterolateral; 7, mid anterior; 8, mid anteroseptal; 9, mid inferoseptal; 10, mid inferior; 11, mid inferolateral; 12, mid anterolateral; 13, apical anterior; 14, apical septal; 15, apical inferior; 16, apical lateral; 17, apex. Basal, segments 1–6; Mid, segments 7–12; Apical, segments 13–16; Anterior, segments 1, 7, 13; Septum, segments 2, 3, 8, 9, 14; Inferior, segments 4, 10, 15; Lateral, segments 5, 6, 11, 12, 16.

TABLE 8 | Left ventricular longitudinal myocardial peak systolic strain values from long axis view.

Segment	Men				Women			
	Age				Age			
	≤44	45–59	≥60	All	≤44	45–59	≥60	All
1	−14.7 ± 7.2	−9.9 ± 5.5	−13.3 ± 8.5	−12.7 ± 7.4	−14.7 ± 8.9	−12.4 ± 7.4	−10.3 ± 6.4	−12.5 ± 7.8
2	−20.5 ± 7.4	−17.3 ± 6.7	−14.6 ± 6.9	−17.5 ± 7.3	−23.3 ± 7.8	−22.2 ± 5.7	−15.8 ± 10.1	−20.4 ± 8.6
3	−19.6 ± 4.9	−16.5 ± 6.4	−18.8 ± 6.2	−18.3 ± 6.0	−17.1 ± 4.7	−20.7 ± 6.8	−15.7 ± 7.3	−17.8 ± 6.6
4	−17.8 ± 6.7	−18.3 ± 8.8	−25.1 ± 7.2	−20.4 ± 8.2	−23.1 ± 9.0	−25.7 ± 8.4	−22.7 ± 9.7	−23.8 ± 9.0
5	−32.0 ± 8.2	−30.0 ± 10.2	−33.9 ± 6.6	−31.9 ± 8.5	−33.2 ± 8.1	−36.1 ± 9.4	−35.2 ± 9.2	−34.9 ± 8.9
6	−28.3 ± 7.9	−34.0 ± 8.7	−30.7 ± 8.5	−30.9 ± 8.6	−32.5 ± 8.0	−36.0 ± 7.7	−36.2 ± 9.6	−34.9 ± 8.5
7	−17.9 ± 8.3	−21.4 ± 10.5	−23.7 ± 11.4	−21.0 ± 10.6	−28.3 ± 9.3	−23.8 ± 11.4	−23.7 ± 11.8	−25.3 ± 11.0
8	−19.6 ± 9.2	−19.3 ± 7.8	−21.6 ± 7.4	−20.2 ± 8.1	−20.9 ± 6.7	−21.3 ± 6.5	−21.5 ± 9.0	−21.3 ± 7.4
9	−23.3 ± 7.9	−22.8 ± 8.5	−24.9 ± 5.9	−23.7 ± 7.5	−24.9 ± 7.2	−23.9 ± 6.5	−24.4 ± 8.5	−24.4 ± 7.4
10	−24.7 ± 8.6	−21.6 ± 7.1	−19.7 ± 9.7	−22.0 ± 8.7	−25.4 ± 10.4	−27.8 ± 8.7	−21.8 ± 9.8	−25.0 ± 9.8
11	−26.4 ± 9.2	−30.9 ± 9.0	−24.7 ± 8.5	−27.3 ± 9.2	−33.2 ± 10.1	−32.7 ± 11.4	−30.1 ± 11.6	−32.0 ± 11.0
12	−25.0 ± 11.6	−24.9 ± 10.9	−26.0 ± 8.5	−25.3 ± 10.3	−30.5 ± 9.2	−32.8 ± 11.2	−32.1 ± 9.2	−31.8 ± 9.8
13	−29.8 ± 7.1	−30.2 ± 8.8	−26.9 ± 10.5	−29.0 ± 8.9	−30.3 ± 8.9	−33.9 ± 10.9	−33.0 ± 11.9	−32.4 ± 10.6
14	−17.7 ± 5.7	−15.7 ± 6.1	−16.1 ± 4.4	−16.5 ± 5.4	−17.2 ± 7.6	−15.2 ± 5.2	−17.6 ± 4.8	−16.7 ± 6.0
15	−27.7 ± 9.9	−24.3 ± 11.4	−17.9 ± 9.0	−23.3 ± 10.8	−24.7 ± 11.1	−17.9 ± 9.7	−21.7 ± 10.0	−21.4 ± 10.5
16	−20.1 ± 7.6	−15.9 ± 6.9	−17.2 ± 5.5	−17.8 ± 6.9	−17.8 ± 10.1	−13.0 ± 7.8	−15.8 ± 7.6	−15.5 ± 8.7
17	−28.0 ± 5.5	−23.1 ± 5.2	−22.5 ± 5.1	−24.5 ± 5.8	−23.9 ± 6.5	−22.1 ± 5.4	−24.0 ± 4.2	−23.3 ± 5.4
Basal	−22.2 ± 2.9	−21.0 ± 3.8	−22.7 ± 4.0	−21.9 ± 3.6	−24.0 ± 3.7	−25.5 ± 3.7	−22.7 ± 4.7	−24.0 ± 4.2
Mid	−22.8 ± 4.4	−23.5 ± 4.6	−23.4 ± 3.6	−23.2 ± 4.2	−27.2 ± 3.6	−27.0 ± 4.2	−25.6 ± 4.5	−26.6 ± 4.1
Apical	−23.8 ± 4.2	−21.5 ± 4.5	−19.5 ± 4.2	−21.6 ± 4.6	−22.5 ± 5.5	−20.0 ± 5.0	−22.0 ± 4.0	−21.5 ± 4.9
Anterior	−20.8 ± 3.3	−20.5 ± 4.1	−21.3 ± 3.8	−20.9 ± 3.7	−24.4 ± 4.0	−23.4 ± 4.3	−22.3 ± 3.7	−23.4 ± 4.1
Septum	−20.1 ± 3.2	−18.3 ± 3.4	−19.2 ± 3.4	−19.2 ± 3.4	−20.7 ± 3.3	−20.7 ± 3.3	−19.0 ± 4.7	−20.1 ± 3.9
Inferior	−23.4 ± 4.3	−21.4 ± 3.9	−20.9 ± 3.9	−21.9 ± 4.1	−24.4 ± 6.9	−23.8 ± 3.7	−22.1 ± 4.2	−23.4 ± 5.1
Lateral	−26.4 ± 3.8	−27.1 ± 4.3	−26.4 ± 3.1	−26.6 ± 3.8	−29.4 ± 3.0	−30.1 ± 4.0	−29.9 ± 4.7	−29.8 ± 3.9

Data are expressed as mean ± SD. 1, basal anterior; 2, basal anterolateral; 3, basal inferoseptal; 4, basal inferior; 5, basal inferolateral; 6, basal anterolateral; 7, mid anterior; 8, mid anteroseptal; 9, mid inferoseptal; 10, mid inferior; 11, mid inferolateral; 12, mid anterolateral; 13, apical anterior; 14, apical septal; 15, apical inferior; 16, apical lateral; 17, apex.

TABLE 9 | Intra-observer and inter-observer variability.

Variable	Variability	Mean bias ± SD	Limits of agreement	Coefficient of variation (%)	ICC (95% CI)
LA E_{ll}	Intra-observer	−0.41 ± 1.24	−2.88 to 2.07	2.43	0.994 (0.985, 0.998)
	Inter-observer	3.97 ± 4.05	−4.12 to 12.06	14.85	0.929 (0.820, 0.972)
LV E_{ll}	Intra-observer	−0.22 ± 0.89	−2.00 to 1.56	3.32	0.966 (0.913, 0.986)
	Inter-observer	−0.07 ± 0.77	−1.61 to 1.47	2.88	0.969 (0.921, 0.988)
LV E_{cc}	Intra-observer	−0.04 ± 0.72	−1.48 to 1.41	2.15	0.990 (0.975, 0.996)
	Inter-observer	−0.44 ± 1.31	−2.18 to 3.05	4.03	0.960 (0.898, 0.984)
LV E_{rr}	Intra-observer	−1.02 ± 14.44	−29.89 to 27.85	11.28	0.793 (0.477, 0.918)
	Inter-observer	4.22 ± 15.04	−25.85 to 34.29	12.54	0.832 (0.576, 0.934)
RV E_{ll}	Intra-observer	−0.54 ± 1.66	−3.86 to 2.78	3.92	0.975 (0.937, 0.990)
	Inter-observer	−4.43 ± 3.18	−10.8 to 1.94	14.85	0.891 (0.726, 0.957)

LA, left atrium; LV, left ventricle; RV, right ventricle; E_{ll}, longitudinal strain; E_{cc}, circumferential strain; E_{rr}, radial strain; ICC, intra-class coefficient; CI, confidence interval.

and longitudinal strains; in addition (iv) it provides quantitative ranges of LA, LV, and RV strain in healthy Chinese stratified into different age groups.

Normal Ranges of Strain

CMR-FT, a technique analogous to echocardiographic speckle tracking, has proven to be a feasible and reproducible approach for quantifying LA dynamics in terms of strain and strain rate (Kowallick et al., 2014, 2015b). Corresponding imaging biomarkers are increasingly recognized as having the potential to predict outcomes in a variety of cardiovascular disease states (Kowallick et al., 2014, 2015a; Inoue et al., 2015; Dick et al., 2017). The basic function reflected by LA strain depends on the use of either the QRS complex (R–R gating) or the P wave at the initiation of the calculation. When the R wave is used, as in this study, the first peak between the R and T waves corresponds to reservoir function (Pathan et al., 2017). Reservoir strain of LA derived from CMR-FT in this study included 32.8 ± 9.2% for longitudinal and 40.3 ± 13.4% for circumferential strain. The longitudinal strain value was lower than 46 ± 13% reported by Dick et al. (2017) from 25 healthy subjects. The lower LA volume in our subjects may explain the difference in E_{ll} values, since it has been reported that deformation parameters from CMR-FT for atrial reservoir functions are strongly related to volumetric indexes (Kowallick et al., 2014).

The LV myocardial E_{ll} and E_{cc} values obtained in our study were similar to the normal ranges of a recently published systematic review and meta-analysis (Vo et al., 2018). Andre et al. (2015) reported FT-derived LV longitudinal strain and circumferential strain values of −21.6 ± 3.2 and −21.3 ± 3.3% in 150 healthy volunteers at 1.5T (Achieva, Philips Medical Systems), which is concordant with our results. Taylor (Taylor et al., 2015), using CMR-FT at 1.5T (Magnetom Avanto, Siemens), reported values of −21.3 ± 4.8 and −26.1 ± 3.8% derived from a group of 100 individuals comprising 10 men and women in each of 5 age deciles. The values are also in close agreement with our study. Relative greater radial strain exhibited in this study than previously results reported in (Andre et al., 2015; Taylor et al., 2015; Vo et al., 2018). It may be partly explained by that in our study, papillary muscles were included in the endocardial borders, which would have resulted in greater LV volume estimates with the consequent higher values for myocardial radial strain. Moreover, feature tracking imaging (FTI) algorithms inherently depend on image quality and endocardial border definition. And the large standard deviation of radial strain (up to 19.4% in this study) may suggest limitation for the present FTI algorithms in evaluating radial strain and advanced algorithms may be warranted. Our results showed regional variations that LV longitudinal and circumferential strain, and strain rate were highest in the lateral walls and lowest in the septum. Peak circumferential strain was lower in the mid-cavity than at the base or apex, which is consistent with results in Taylor et al. (2015).

The RV longitudinal strain measured in our study was consistent with that of Levy et al. (2014) who reported a range of −20.8 to −34.1% (mean, −29.0%; 95% CI, −31.5 to −26.5%) based on a meta-analysis of two-dimensional speckle tracking echocardiographic-derived right ventricular strain in children. Our values for RV longitudinal strain were lower than those of Truong et al. (2017) and Liu et al. (2018), both post-processing with Circle Cardiovascular Imaging Tissue Tracking software. RV strains were obtained from 50 consecutive patients with no identified cardiac pathology (−22.1 ± 3.5%; ages 4–81 years; median age, 32 years) in Truong (Truong et al., 2017), and 100 healthy subjects containing 10 males and 10 females from each decade (−21.9 ± 3.24%; ages 20–70 years) (Liu et al., 2018). CMR-FT is based on the features at the myocardial boundary voxels and RV strain assessment software only measures endocardial strain, while Truong and Liu used both endocardial and epicardial borders to determine the myocardial deformable model. Secondly, RV strain assessment in our study included the septal values, as CMR-FT utilizes a LV tracking program, and Truong et al. (2017) only assessed the free wall without the inter-ventricular septum. More importantly, compared to CMR tissue tracking (CMR TT) based on the myocardium (Truong et al., 2017), manual intervention was needed to correct inaccurate tracking results in the feature tracking software, which could potentially introduce inconsistencies arising from image noise and the complex anatomical structure along the boundary. It would seem reasonable that differences in feature tracking

software and strain assessment methods, a very thin free wall and presence of heavy trabeculations, variability among subjects, all combined to produce different values of RV longitudinal strain.

Gender and Age Specific of Cardiac Deformation

Conflicts remain regarding the effects of gender and age on cardiac deformation. Vo et al. (2018) showed no association of LV E_{ll}, E_{rr} and RV E_{ll} with age, gender, software, field strength, sequence, LVEF or LV size. However, most published studies, such those as by Lawton et al. (2011), Augustine et al. (2013), and Taylor et al. (2015) reported greater strain in females, which is consistent with the trend in our results.

The LA E_{ll}, E_{cc} and LV E_{cc}, E_{rr} exhibited age dependency, although the correlation was weak. Systolic strain declined with increasing age. Higher correlation of LA strain with age compared to LV strain suggests greater clinical impact of age on LA strain. In contrast, age-related LV stiffness associated with a decline in diastolic function could be compensated for by increases in systolic wall thickening, thereby explaining the increase in radial strain with age. While our findings conflict with the result in Taylor et al. (2015), who reported an age-related increase in circumferential strain, our findings concur with those from Kuznetsova et al. (2008) and Dalen et al. (2010) who reported a decline in longitudinal strain associated with age. The discordance may be due to complexities in the course of aging rather than simply age and gender, hence the statistically significant but weak effects of age in our results.

Finally, we did not observe a significant association between age and RV longitudinal strain. This is consistent with the results from previous studies using speckle tracking echocardiography (Levy et al., 2014) and CMR tissue tracking (Truong et al., 2017; Liu et al., 2018; Vo et al., 2018).

Reproducibility

In this study, acceptable intra- and inter-observer agreement was found for peak systolic strain of LA, LV, and RV. Reproducibility obtained for LV E_{cc} was the best, followed by E_{ll} and then E_{rr}. This is consistent with the findings of Taylor et al. (2015). It suggested that FTI allowed for reproducible quantification of systolic strains. And validation of LV E_{rr} is more challenging as this is generally less accurately quantified by all deformation algorithms.

Clinical Perspective

CMR-FT derived wall motion assessment reliably quantifies LA, LV, and RV strain and SR from standard SSFP cine images. It seems a promising approach for the study of physiology in health and disease states. CMR-FT based global and segmental deformation quantification helps early detecting of subclinical myocardial dysfunction, monitoring the progress and predicting the outcome of the disease, such as heart failure with preserved ejection fraction (Zhong et al., 2013). Quantitative assessment of systolic right ventricular myocardial deformation can be used as a more quantitative tool to measure RV function in diseases such as repaired tetralogy of Fallot (Zhong et al., 2012) and pulmonary arterial hypertension (Leng et al., 2016).

Limitations of Study

Given the higher prevalence of risk factors such as diabetes mellitus, atherosclerosis, hypertension, and hyperlipidemia among the very elderly subjects, future studies may be required to focus on the normal ranges for these groups after stratification by risk factors. Other risk factors such as smoking, blood pressure, alcohol consumption are also missing in the present study, and how they will affect the strain values needs further investigation. Right atrial deformation was excluded from this analysis, as border tracking of the right atrium is known to be technically challenging owing to thin walls and the potential for morphological variations. This may be a contributing factor to the current lack of consensus on the clinical value of right atrial strain analysis.

AUTHOR CONTRIBUTIONS

XM, ML, and LZ conceived and designed the study. JP, XZ, LZ, ZF, ZW, HC, SL, R-ST, and AK performed the experiments. JP wrote the paper. XM, ML, LZ, ZF, XZ, and JA reviewed and edited the manuscript. All authors read and approved the manuscript.

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Effect of Endurance and Strength Training on the Slow Component of $\dot{V}O_2$ Kinetics in Elderly Humans

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We compared the effects of 8 weeks of high intensity, aerobic interval training (*HIT)* and isoinertial resistance training (*IRT)* on: (i) $\dot{V}O_2$ kinetics during heavy (*HiEx*) intensity exercise and; (ii) work economy during moderate (*ModEx*) intensity exercise in 12 healthy elderly men (69.3 ± 4.2 years). Breath-by-breath $\dot{V}O_2$ and muscle deoxygenation ([*HHb*] by means of *NIRS*) were measured in *HiEx* and *ModEx* at identical workloads before and after trainings. In *HiEx,* $\dot{V}O_2$ and *HHb* responses were modeled as tri-exponential and mono-exponential increasing functions, respectively. A two-way ANOVA for repeated measures analysis was made; Effect size (η^2) was also evaluated. After *HIT* the amplitude and the time delay of the slow component of O_2 uptake ($\dot{V}O_{2sc}$) during *HiEx* were smaller (−32%; $P = 0.045$) and longer (+19.5%; $P = 0.001$), respectively. At *Post IRT*: (i) during *ModEx, gain* was lower (−5%; $P = 0.050$); (ii) during *HiEx*, τ_2 (+14.4%; $P = 0.050$), d_3 (+8.6%; $P = 0.050$), and τ_3 (+17.2%; $P = 0.050$) were longer than at *Pre IRT*. After *HIT,* the decrease of the $\dot{V}O_{2sc}$ amplitude was likely induced by the beneficial effects of training on a more responsive O_2 delivery and consumption cascade leading to a better muscle metabolic stability. *IRT* training was able to increase exercise economy during *ModEx* and to reduce the amplitude and delay the onset of $\dot{V}O_{2sc}$ during *HiEx*. These effects should be due to the reduction and the delayed recruitment of Type II muscle fibers. The better exercise economy and the delayed appearance of $\dot{V}O_{2sc}$ induced by *IRT* suggests that strength training might be included in endurance training programs to improve exercise economy and resistance to fatigue in this population of old subjects.

Keywords: high intensity interval training, isoinertial strength training, heavy intensity exercise, near-infrared spectroscopy, oxygen uptake kinetics, elderly, muscle strength, slow component

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INTRODUCTION

The kinetics of alveolar O_2 uptake ($\dot{V}O_{2A}$) upon the onset of constant work rate (*CWR*) exercise of moderate intensity (*ModEx*) is usually described by a double exponential model (Poole and Jones, 2012). The first, rapid component – phase I – is characterized by a short time constant and it is caused by the prompt increase of cardiac output at the beginning of exercise. The second component – phase II – is considered to be a reliable proxy of muscular O_2 uptake and is characterized by a time constant of about 20 s in young, healthy, and trained subjects (Poole and Jones, 2012).

During heavy intensity exercise (*HiEx*), i.e., above the lactic threshold (*LT*), the attainment of the steady state oxygen consumption ($\dot{V}O_{2ss}$) is delayed due to the presence of a slow increase of $\dot{V}O_2$($\dot{V}O_{2sc}$) that starts about 150–200 s after the onset of exercise (Jones et al., 2011). Furthermore, if the exercise is performed in the very heavy domain (*VHiEx*), e.g., above the so called critical power, $\dot{V}O_{2ss}$ cannot even be attained, since $\dot{V}O_2$ keeps increasing up to $\dot{V}O_{2max}$, a condition that heralds the interruption of exercise (Poole and Jones, 2012).

From the performance standpoint, $\dot{V}O_{2sc}$ is important, as it is related to increased susceptibility to fatigue: $\dot{V}O_{2sc}$ amplitude, e.g., is linearly related to the time to fatigue in obese adolescents (Salvadego et al., 2010).

There is compelling evidence that muscular mechanisms are largely responsible for $\dot{V}O_{2sc}$ (Poole et al., 1991) and several data support the notion that the progressive recruitment of Type II muscle fibers during *HiEx/VHiEx* exercise is the main determinant of $\dot{V}O_{2sc}$ (Poole and Jones, 2012). Type II fibers are characterized by a higher *ATP* cost of force production (Stienen et al., 1996) and by higher O_2 consumption for *ATP* synthesis (Willis and Jackman, 1994) than Type I fibers and it has been also demonstrated that $\dot{V}O_{2sc}$ is more evident in humans with a higher percentage of Type II fibers (Barstow et al., 1996). Recent findings, however, have somehow challenged this view suggesting that the progressive recruitment of the less economic Type II fibers is not strictly necessary to induce $\dot{V}O_{2sc}$. Conversely, $\dot{V}O_{2sc}$ may be caused by events occurring inside the recruited fibers (Zoladz et al., 2008).

In addition, it has been shown that $\dot{V}O_{2sc}$ can be modulated by manipulations of O_2 delivery (Poole and Jones, 2012). Therefore, decreased O_2 availability may affect the $\dot{V}O_{2sc}$ of individuals in whom local O_2 delivery during exercise is impaired (e.g., healthy aging) and a clear mismatch between O_2 delivery and consumption is present (Murias et al., 2010a,b).

The effects of physical training have been explored to disclose the mechanisms underpinning $\dot{V}O_{2sc}$ (Jones et al., 2007). Endurance training improves the so-called metabolic stability, leading to a lower decrease in phosphocreatine concentration *[PCr]* and a diminished intramuscular acidosis during *HiEx* in connection with a less evident $\dot{V}O_{2sc}$ (Poole and Jones, 2012). Since low levels of intramuscular *[PCr]* and of *pH* characterize *HiEx/VHEx* exercise (Jones et al., 2008, 2011), these results seem to suggest that the slow decrease in *[PCr]* and increase of $[H^+]$ occurring at these exercise intensities (Jones et al., 2008) are the main mechanistic determinants of $\dot{V}O_{2sc}$. In addition, endurance training improves metabolic hyperemic response and optimizes the matching between local O_2 delivery and utilization, especially in individuals with suboptimal vascular response, such as elderly subjects (Murias et al., 2010a,b). Therefore, the correlation between the indexes that describe amelioration of local peripheral perfusion and the attenuation of the amplitude of $\dot{V}O_{2sc}$ might suggest a potential mechanistic link between O_2 delivery and $\dot{V}O_{2sc}$.

Also, strength training, by decreasing the number of motor units (MUs) recruited at the same work rate (*WR*), may theoretically attenuate $\dot{V}O_{2sc}$, as a smaller number of less economic Type II fibers would be recruited at the same *WR*. However, this hypothesis has been somehow disproved in young adults in whom isometric strength training failed to abate the amplitude of $\dot{V}O_{2sc}$ (Zoladz et al., 2012). Yet, more effective strength training modalities applied to subjects with large muscular strength deficits may potentially elicit more evident and beneficial effects on $\dot{V}O_{2sc}$ via this mechanism.

Finally, it has also been suggested that strength training may improve mechanical efficiency during *ModEx* (Beattie et al., 2014). From the practical standpoint, a greater exercise economy associated with the attenuation of $\dot{V}O_{2sc}$ induced by strength training may ameliorate exercise capability in subjects characterized by a low exercise capacity.

Therefore, we studied in a group of healthy, moderately active elderly men the effect of high intensity interval training (HIT) and isoinertial strength training (*IRT)* on: (1) $\dot{V}O_2$ kinetics and muscular oxygenation of the exercising muscle by near-infrared spectroscopy (*NIRS*) during cycling *HiEx* performed at the same absolute *WR* before and after training; (2) work economy during *ModEx* at the same absolute WR. In addition, (3) Muscle cross sectional area (*CSA*) and muscle volume (*Vol*) of the quadriceps; and (4) muscular strength were assessed. We analyzed these data to determine the effects and relative mechanisms induced by *HIT* and *IRT* on the entity of $\dot{V}O_{2sc}$.

MATERIALS AND METHODS

Subjects

Twelve moderately active Caucasian men (mean ± SD; 69.3 ± 4.2 years, range, 65–75; 77.8 ± 10.4 kg; height 1.72 ± 0.05 m) volunteered to participate in the study. A medical examination, to determine exclusion criteria, and a cycle-ergometer stress test, to exclude abnormal responses to intense exercise, were preliminarily performed. The study protocol was approved by the institutional review board (approval on June 18th, 2013) and designed in accordance with ethical standards, the provisions of the Declaration of Helsinki and national and international guidelines. Written informed consent was obtained from each subject before the study.

Experimental Design

A two-factor within-subject design (A × B × S) (Keppel and Wickens, 2004) was used in which each subject (factor, S) received all the combinations that originated by crossing the two factors A and B. One fixed factor (A) was training modality (levels: *HIT* and *IRT*); the second fixed factor (B) was time (levels: *Pre* and *Post* training). The subjects were evaluated immediately before (*Pre HIT*) and immediately after 8 weeks of *HIT* (*Post HIT*). Then, after 4 months of recovery during which the subjects were asked to keep the same habitual lifestyle (**Figure 1**), the subjects were evaluated again before (*Pre IRT*) and immediately after 8 weeks of *IRT* (*Post IRT*). Before the first data collections, a familiarization session was conducted.

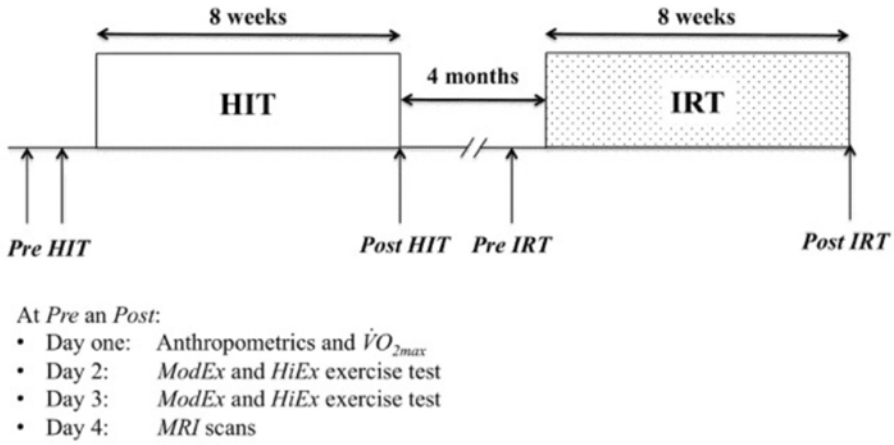

FIGURE 1 | Schematic representation of the experimental design.

MRI scans for measuring muscle *CSA* and *Vol* were obtained before and after *HIT* and *IRT*.

Tests were performed in the morning on four consecutive days: the main anthropometrical data and $\dot{V}O_{2\,max}$ were measured on day 1; *CWR ModEx* and *HiEx* exercise tests were performed on days 2 and 3; *MRI* scans were obtained on day 4.

Training Protocols

- *High intensity interval training (HIT).* The subjects trained three times a week for 8 weeks. Training consisted of seven 2-min bouts of cycling (915 E, Monark, Varberg, Sweden) at about 85–95% of individual $\dot{V}O_{2\,max}$ interspersed by 2 min of recovery at about 40% of $\dot{V}O_{2\,max}$. Each series was preceded by 10 min of active warm-up.
- *Isoinertial resistance training (IRT).* Resistance exercise was performed on a seated knee extension flywheel (4.2 kg) ergometer (YoYo Technology AB, Stockholm, Sweden) three times a week for 8 weeks. Each session consisted of four sets of seven maximal, coupled concentric extensions and eccentric flexions of the knee. The sets were interspersed by 3-min of rest and initiated immediately after performing two submaximal actions. Each exercise session was preceded by 10 min of active warm-up.

Anthropometry

Body weight (*BW*) and stature were measured with a Tanita electronic scale BWB-800 MA (Tanita, Arlington Heights, IL, United States) and a stadiometer (Holtain Ltd., Crymych, Pembs. United Kingdom).

Maximal Oxygen Uptake, Ventilatory Thresholds

All cycling tests were performed on an electromechanically braked cycle ergometer (Excalibur Sport, Lode, Netherlands) operated by a personal computer connected to a metabolic cart. Breath-by-breath gas exchanges were measured continuously at the mouth with a metabolic cart (Quark b^2, Cosmed, Rome, Italy) that was calibrated following the manufacturer's instructions before each experiment.

$\dot{V}O_{2\,max}$ and ventilatory thresholds were measured during a ramp test (Poole et al., 2008) and a supra maximal *CWR* test following the procedure illustrated by Bruseghini et al. (2015).

Responses to Moderate Intensity and Heavy-Intensity Exercise

Responses to *ModEx* and *HiEx* exercise were evaluated at a *WR* corresponding to 90% of individual gas exchange threshold (*GET*) and to about 50% of the difference between *GET* and respiratory compensation point (*RCP*) determined at *Pre HIT*. The *WR* was calculated using the linear regression of $\dot{V}O_2$ vs. *WR* considering the lag of the $\dot{V}O_2$ increase with respect to that of the workload determined at the ramp test and it was maintained constant in all the sessions. After instrumentation and preparation, the subjects rested on the cycle ergometer for 3 min before starting to pedal for 3 min at 30 W; then, *WR* was increased to the preselected *WR* and maintained for 6 min. The procedure was repeated three times (twice for *ModEx* and once for *HiEx*) with 10 min of recovery between each test. The entire procedure was repeated the following day. Pedaling frequency was strictly maintained between 70 and 80 revolutions per minute by the aid of a visual pacemaker.

Muscle Oxygenation

Vastus lateralis muscle oxygenation during *HiEx* was evaluated by means of a frequency-domain-multidistance *NIRS* system (OxiplexTS, ISS, Champaign, IL, United States) that provided continuous measurement of absolute concentrations (μM) of oxyhemoglobin (*[O_2Hb]*) and deoxyhemoglobin (*[HHb]*) (De Roia et al., 2012). The thickness of the skin and of the subcutaneous fat layer of the explored area was assessed by ultrasound (ACUSON P50 ultrasound system, Siemens, Erlangen, Germany) and it ranged from 6.4 to 11.8 mm, 8.1 mm ± 1.5. In addition, cutaneous landmarks were pen-marked on a transparent acetate sheet placed on the area of the probe

so that it could be applied on the same site in the subsequent experimental sessions.

Muscular Strength and Morphology

Knee extension torque (T_k) of the dominant limb was evaluated with an isokinetic dynamometer (CMSi Cybex Humac Norm Dynamometer, Stoughton, MA, United States) during concentric contractions at 60° s^{-1} and 120° s^{-1} angular speeds. The subjects went through several practice trials and performed contractions while seated on the reclining chair of the dynamometer. The lower part of the leg was strapped to the end of the lever arm and the center of rotation of the knee was aligned with the axis of the dynamometer. Before the test, the subjects completed 10-min of warm-up exercise on a stationary bike. Three maximal trials were performed for each condition with 3 min of recovery between each trial. The highest T_k values (as peak values) were recorded for further analysis.

MRI scans were obtained after 1 h of supine rest to avoid the influence of posture-related fluid shifts on muscle size following the procedure illustrated by Bruseghini et al. (2015).

Data Analysis

Breath-by-breath $\dot{V}O_2$ values were interpolated to 1 s intervals, time aligned with the onset of exercise transition, and treated by subtracting the $\dot{V}O_2$ steady state value (average values of the last 30 s of trial) at 30 W. The data from the trials were then combined to obtain a single data file for each subject and condition.

$\dot{V}O_2$ kinetics during *HiEx* exercise was modeled as a sum of three exponential increasing functions:

$$\begin{aligned}\dot{V}O_2 = {} & U(t-d_1)\times(A_1\times(1-e^{-(t-d_1/\tau_1)}))+\\ & U(t-d_2)\times(A_2\times(1-e^{-(t-d_2/\tau_2)}))+\\ & U(t-d_3)\times(A_3\times(1-e^{-(t-d_3/\tau_3)}))\end{aligned} \quad (1)$$

where τ_1, τ_2, and τ_3 are the time constants of the exponential increases during phase I, phase II, and phase III (the slow component), d_1, d_2, and d_3 are the time delays and A_1, A_2, and A_3 are the asymptotic amplitudes of the corresponding phases. $U(t-d)$ is the unit step function defined as:

$$U(t-d)\begin{cases}0 \text{ if } t<d\\ 1 \text{ if } t\geq d\end{cases} \quad (2)$$

The value of the amplitude at the end of phase I, (A'_1), which terminated at the start of phase II, was calculated as (2):

$$A_1^{'} = A_1\times\left(1-e^{-(d_2/\tau_1)}\right) \quad (3)$$

Note that the first addend of Eq. (1) is truncated as it reaches A'_1 at $t = d_2$, and doesn't continue to rise toward its asymptotic value A_1. The physiologically significant amplitude of the primary exponential (A'_2) was defined as the sum of $A'_1 + A_2$ (Barstow et al., 1996). Because of the uncertain validity of the asymptotic value of A_3, we used the value of the amplitude of the slow component at the end of the exercise (A'_3) (Barstow et al., 1996). The change of O_2 uptake from the $\dot{V}O_2$ steady state value at 30 W and the values of $\dot{V}O_2$ at A'_3 ($\Delta\dot{V}O_2EE$) was given by $A'_2 + A'_3$. To compare the subjects working at different absolute workloads, the gain in the primary response ($G_{Prim} = A'_2/\Delta WR$) and the gain in the total response at the end of *HiEx* exercise [$G_{Tot} = (A'_2 + A'_3)/\Delta WR$] were calculated. The relative contribution of the slow component to the overall $\dot{V}O_2$ response was calculated as $A'_3/(A'_2 + A'_3)$.

The gain (G) during *ModEx* exercise was calculated as the ratio between net steady state $\dot{V}O_2$ and the corresponding net increase of WR ($G = A'_2/\Delta WR$).

NIRS derived *[HHb]* response during *HiEx* was first interpolated to 1-s intervals, then time aligned with the onset of exercise transition and finally treated by subtracting the steady state value at 30 W. Then, the fitting window was constrained from the start of exercise to the onset of the slow component of *[HHb]* (Breese et al., 2013). Mean response time (*MRT*) was calculated as the sum of τ_1 and d_1. The primary *[HHb]* amplitude was divided by the phase II asymptotic amplitude A_2 to yield the $\Delta[HHb]/\Delta\dot{V}O_2$: it was considered as an index of the increase in fractional muscle O_2 extraction required to sustain a given net increment in $\dot{V}O_2$ during the primary phase (Murias et al., 2014). The net increase from the baseline of the values of *[HHb]* and of *[O_2Hb]* after 120 s of exercise and at the end of the exercise were calculated over 30 s time windows, the first interval of time being centered on the 120th-second and second interval including the last 30 s of exercise.

The net increases in *[HHb]* and in *[O_2Hb]* were then added to obtain the net increase in total hemoglobin concentration (*[Hb_{tot}]*) in the volume of tissue explored by the probe. *[Hb_{tot}]* was only calculated at 120 s of exercise and at the end of exercise.

The parameters of the $\dot{V}O_2$ models were estimated by means of an iterative, weighted non-linear least-squares procedure (Marquardt, 1963) that was developed in G-Language (LabVIEW 7.0, National Instruments, Austin, TX, United States). Initial guesses of the parameters of the model were entered after visual inspection of the data. The 95% confidence intervals of the τ_2 and τ_3 of $\dot{V}O_2$ kinetics and of τ_1 of *HHb* kinetics were generated by means of Monte Carlo simulation (Motulsky and Christopoulos, 2004) using commercial software for data analysis (GraphPad Prism version 6.00 for Macintosh, GraphPad Software, La Jolla, CA, United States). Amplitudes and time delays were constrained to the best-fit values and the time constants were allowed to vary.

MRI scans were transferred electronically from the scanner to a personal computer (Macintosh mac Book Pro, Apple, Cupertino, CA, United States) and analyzed with OsiriX (version 3.7.1 32 bit) by using manual planimetry to calculate *CSA* and *Vol* of the quadriceps of the dominant leg (Bruseghini et al., 2015). The same investigator carried out all measurements. The reliability of this measurement was assessed over five separate measurements of the *CSA* of three heads of the quadriceps muscle taken distally at 50% of the femur bone length; the average coefficient of variation of measuring the same image was 0.92% for total *quadriceps femoris*.

Statistical Analysis

All values in the text and the tables are presented as mean ± SD. Two-factor within-subject ANOVA analysis for repeated measures was carried out according to Keppel and

Wickens (2004): (i) *F* values were calculated taking into account the possible violation of sphericity as suggested by Geisser and Greenhouse; (ii) single contrasts within subjects (time, *Pre* vs. *Post*) and between subjects (Training, *HIT* vs. *IRT* and interactions were computed; (iii) effect size was evaluated with partial squared correlation factor or η^2, (η^2_w, η^2_b, η^2_{inte}, suffix are related to within, between, and interactions analysis) which expresses the ratio between explained variability and total variability in the population, but compensates for the size of the other treatment effect (either time or training); (iv) effect size (*d*) of the differences between the contrasted values was calculated. Calculations were carried out using an Excel spreadsheet (MO 2010, Microsoft Corp., Seattle, WA, United States) prepared for this purpose. Model 2 linear regressions between bivariate data were calculated according to the method of Deming (Motulsky and Christopoulos, 2004). Correlation between variables was computed using Spearman's correlation coefficient.

Statistical analysis was made by a two-way ANOVA for repeated measures; Effect size was evaluated with partial squared correlation factor or η^2. *P* was always set <0.05.

RESULTS

The data concerning $\dot{V}O_{2\,max}$, ventilatory threshold and muscular strength and mass have been already published in a paper that described the effects of *HIT* and *IRT* on several risk factors of cardiometabolic diseases and on the exercise capability in healthy elderly subjects (Bruseghini et al., 2015). The readers are kindly asked to refer to the indicated paper for further details. Here, only the essential results useful for supporting and discussing the hypothesis related to the present investigation will be summarized.

Briefly, absolute $\dot{V}O_{2\,max}$ increased only after *HIT* (*Pre HIT* 2.34 ± 0.35 *Post HIT* 2.48 ± 0.38 L min^{-1} *P* = 0.015; *d* = 0.83; 95% CI_{Diff}: 0.04 L min^{-1}/0.22 L min^{-1}), with no differences after IRT (*Pre IRT* 2.43 ± 0.43 *Post IRT* 2.44 ± 0.42 L min^{-1}). $\dot{V}O_{2RCP}$, expressed as percent of $\dot{V}O_{2\,max}$, was greater at *Post HIT* (*P* = 0.014; *d* = 0.85; 95% CI_{Diff}: 2.1%/11.1%) and at *Pre IRT* (*P* = 0.007; *d* = 0.96; 95% CI_{Diff}: 3.9%/16.2%) than at *Pre HIT* and it was greater at *Post IRT* than at *Post HIT* (*P* = 0.001; *d* = 1.24; 95% CI_{Diff}: 1.4%/3.8%). *Post hoc* contrast analysis showed that *CSA* and *Vol* were increased after *HIT*: *Vol*, *Pre HIT*: 820 ± 199 cm^3, *post HIT*: 866 ± 199 cm^2; *P* = 0.002; *d* = 1.17; 95% CI_{Diff}: 22.9 cm^3/67.9 cm^3) and after *IRT Vol*, *Pre IRT*: 813 ± 184 cm^3, *post IRT*: 852 ± 188 cm^2; *P* = 0.01; *d* = 0.90; 95% CI_{Diff}: 13.9 cm^3/64.6 cm^3). Finally, maximal isokinetic torque was increased only after *IRT*: T_k 60° s^{-1}, *Pre HIT*: 159.8 ± 24.5 N m, *post HIT*: 163.3 ± 22.2 N m; *P* = 0.360; *d* = 0.27; 95% CI_{Diff}: -3.9 N m/10.9 N m; T_k 60° s^{-1}, *Pre IRT*: 162.4 ± 25.8 N m, *post IRT*: 179.0 ± 31.1 N m; *P* = 0.001; *d* = 1.27; 95% CI_{Diff}: 9.0 N m/24.1 N m.

Response to *ModEx*

The average *CWR* was 72.5 ± 16.3 W in the *ModEx* condition, corresponding to 35–40% of $\dot{V}O_{2\,max}$, i.e., *<GET*. *G* at *Pre HIT* and at *Post HIT* was not significantly different (12.1 mL min^{-1} W^{-1} ± 1.5 vs. 12.4 mL min^{-1} W^{-1} ± 1.0). Conversely, at *Post IRT* (12.0 mL min^{-1} W^{-1} ± 1.0) *G* turned out to be significantly smaller (*P* = 0.049; *d* = 0.63; 95% CI_{Diff}: −0.05 mL min^{-1} W^{-1}/−1.1 mL min^{-1} W^{-1}) than at *Pre IRT* (12.6 mL min^{-1} W^{-1} ± 0.9).

Response to *HiEx*

The average *CWR* was 144.3 ± 26.6 W in the *HiEx* condition and it corresponded approximately to 67–71% of $\dot{V}O_{2\,max}$, i.e., *>GET*, but *<RCP*. The parameters describing the kinetics of $\dot{V}O_2$ and the *NIRS* signals obtained in the *HiEx* condition before and after *HIT* and *IRT* are presented in **Tables 1**, **2**, respectively; **Figures 2A–D** demonstrates the kinetics of $\dot{V}O_2$ at the onset of *HiEx* after *HIT* and *IRT* in a typical subject, respectively. **Figures 2E–H** shows the kinetics of *[HHb]* after *HIT* and *IRT*, respectively.

The amplitude A'_1 of phase I at *Post IRT* was significantly larger than at *Pre IRT* (*P* = 0.018; *d* = 0.81; 95% CI_{Diff}: 0.03 L min^{-1}/0.20 L min^{-1}); the latter value was also significantly smaller than at *Pre HIT* (*P* = 0.029; *d* = 0.72; 95% CI_{Diff}: 0.03 L min^{-1}/0.24 L min^{-1}). The time delay in phase I at *Post IRT* was significantly shorter than at *Post HIT* (*P* = 0.036; *d* = 0.69; 95% CI_{Diff}: −0.3 s/−2.3 s) (**Table 1**).

A'_2 was greater at *Post IRT* than at *Pre IRT* (*P* = 0.028; *d* = 0.73; 95% CI_{Diff}: 0.01 L min^{-1}/0.12 L min^{-1}). The time constant of the primary phase of $\dot{V}O_2$ kinetics (τ_2) during *HiEx* was significantly longer at *Post IRT* than before strength training (*P* = 0.010; *d* = 0.90; 95% CI_{Diff}: 1.5 s/6.9 s) and after *HIT* (*P* = 0.010; *d* = 1.02; 95% CI_{Diff}: 2.2 s/7.9 s). In addition, a significant interaction between training types and time at τ_2 was noted (*P* = 0.010; *d* = 0.94; 95% CI_{Diff}: 2.6 s/13.2 s). This further suggests that *IRT* was specifically able to induce the deceleration of the primary phase of $\dot{V}O_2$ kinetics during *HiEx*. Finally, d_2 at *Pre IRT* was shorter than before *HIT* (*P* = 0.004; *d* = 1.04; 95% CI_{Diff}: −2.5 s/−8.8 s) (**Table 1**).

After *HIT* A'_3 was significantly smaller (*P* = 0.045; *d* = 0.65; 95% CI_{Diff}: −0.01 L min^{-1}/−0.11 L min^{-1}) and d_3 was larger (*P* = 0.001; *d* = 1.55; 95% CI_{Diff}: 19.6 s/43.1 s) than at *Pre HIT* (**Table 1** and **Figures 3A,B**). The relative contribution of the slow component to the overall $\dot{V}O_2$ response [$A'_3/(A'_2 + A'_3)$] (**Figure 3D**) was significantly smaller at *Post HIT* than at *Pre HIT* (*P* = 0.018; *d* = 0.80; 95% CI_{Diff}: −1.4%/−9.2%). Also, *IRT* affected d_3 (**Figure 3B**), as it was longer at *Post IRT* than at *Pre IRT* (*P* = 0.039; *d* = 0.67; 95% CI_{Diff}: 2.0 s/25.3 s). In addition, a significant interaction between training types and time on d_3 was observed (*P* = 0.022; *d* = 0.87; 95% CI_{Diff}: 6.1 s/39.5 s), indicating that *HIT* induced a more marked effect than *IRT* on d_3 (**Figure 3B**). Finally, τ_3 was significantly greater at *Post IRT* than at *Post HIT* (*P* = 0.003; *d* = 1.107; 95% CI_{Diff}: 10.7 s/34.1 s) (**Figure 3C**).

Training did not affect the parameters describing the increase in *HHb* at the onset of *HiEx* exercise (**Table 2**): no changes in A_1, d_1, τ_1, and *MRT* were observed after either *HIT* or *IRT* as compared with the pre-training conditions.

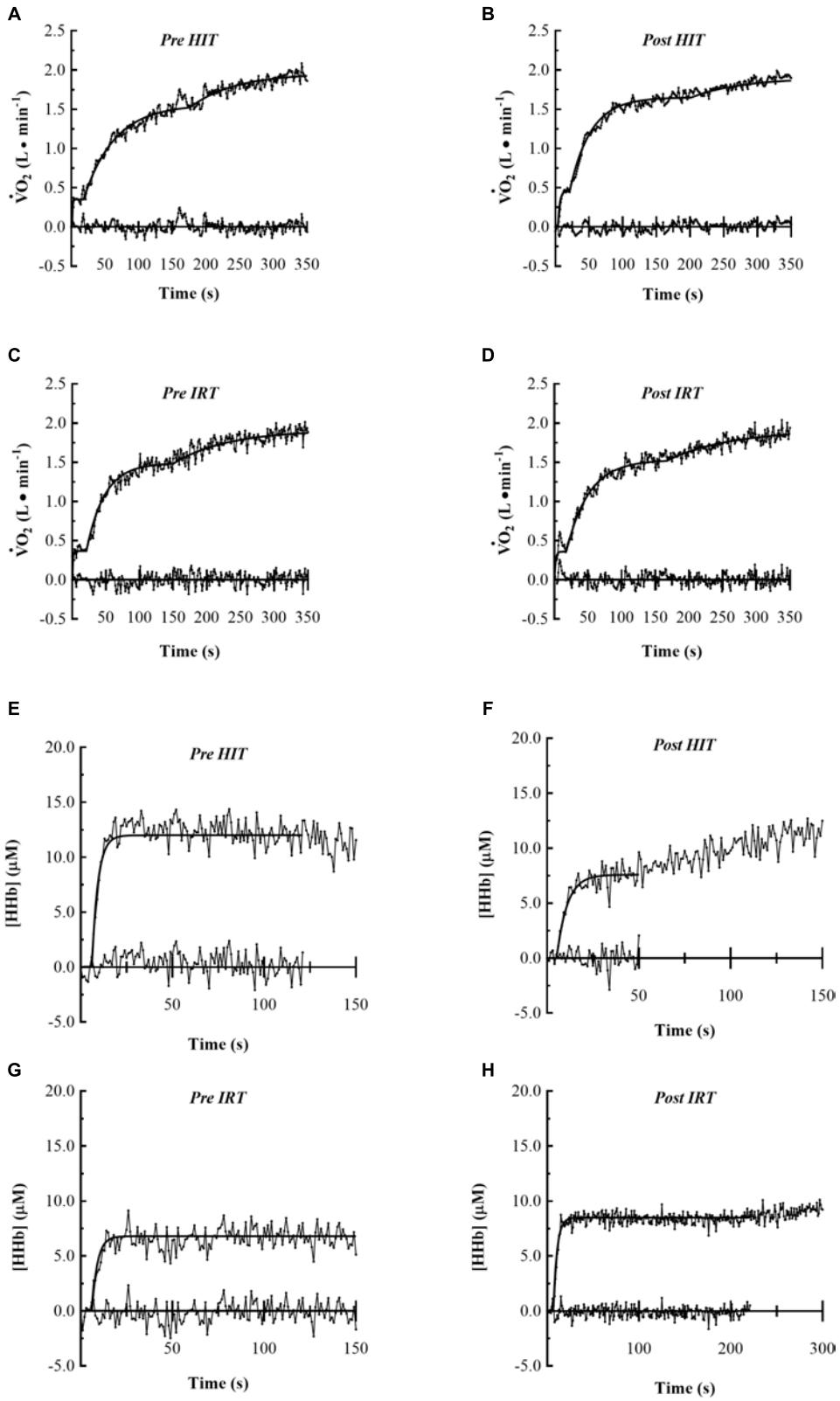

FIGURE 2 | Pulmonary $\dot{V}O_2$ and muscle *HHb* kinetics of typical subjects at the onset of constant work rate exercise of heavy intensity are represented. The first four panels show the following $\dot{V}O_2$ kinetics: *Pre HIT* **(A)**, *Post HIT* **(B)**, *Pre IRT* **(C)** and *Post IRT* **(D)**. The last four panels show the muscle *HHb* kinetics: *Pre HIT* **(E)**, *Post HIT* **(F)**, *Pre IRT* **(G)** and *Post IRT* **(H)**. Data are displayed on 1 s base and the residual plot is shown on *x*-axis.

TABLE 1 | Mean values (SD) of the parameters describing $\dot{V}O_2$ kinetics at the onset of *CWR* exercise of heavy (*HiEx*) intensity.

Parameter				Training			
				HIT		IRT	
	P_b; η^2_b	P_w; η^2_w	P_{int}; η^2_{inter}	Pre	Post	Pre	Post
A'_1 (L min^{-1})	0.061; 0.271	0.098; 0.233	0.248; 0.123	0.37 (0.15)	0.37 (0.20)	0.23* (0.18)	0.35‡ (0.10)
τ_1 (s)	0.006; 0.508	0.051; 0.305	0.535; 0.036	2.1 (1.5)	3.9 (3.2)	1.0* (0.7)	2.0 (1.9)
d_1 (s)	0.024; 0.341	0.213; 0.136	0.264; 0.112	1.3 (1.5)	2.3 (2.7)	0.5 (1.1)	0.5† (0.5)
A'_2 (L min^{-1})	0.101; 0.227	0.025; 0.381	0.751; 0.009	1.45 (0.27)	1.53 (0.22)	1.51 (0.22)	1.58‡,(0.34)
τ_2 (s)	0.227; 0.131	0.410; 0.063	0.013; 0.444	27.7 (7.0)	24.9 (4.3)	25.7 (3.7)	30.0‡,† (5.1)
95% *IC* τ_2 (s)				26.1–29.4	23.9–25.9	24.2–27.1	27.9–30.9
d_2 (s)	0.006; 0.508	0.249; 0.119	0.687; 0.015	17.7 (3.1)	19.3 (8.7)	12.1* (5.0)	14.9 (3.8)
A'_3 (L min^{-1})	0.328; 0.088	0.019; 0.407	0.844; 0.004	0.19 (0.10)	0.13* (0.07)	0.21 (0.12)	0.16 (0.10)
τ_3 (s)	0.003; 0.570	0.411; 0.062	0.127; 0.199	71.0 (8.5)	69.6 (18.4)	81.5 (16.7)	92.0† (15.6)
95% *IC* τ_3 (s)				70.3–71.8	69.4–71.2	78.6–81.7	90.0–94.7
d_3 (s)	0.851; 0.003	0.000; 0.742	0.047; 0.313	165.8 (10.1)	197.1* (13.6)	172.2 (43.2)	185.8‡ (42.6)
$\Delta\dot{V}O_{2EE}$ (L min^{-1})	0.015; 0.433	0.398; 0.07	0.665; 0.018	1.62 (0.30)	1.67 (0.27)	1.72 (0.27)	1.74 (0.34)
$A'_3/(A'_2 + A'_3)$ (%)	0.329; 0.067	0.010; 0.471	0.706; 0.013	13.8 (7.5)	8.5* (3.8)	14.8 (9.5)	10.8 (6.6)
G_{Prim} (mL min^{-1} ΔW)	0.081; 0.251	0.040; 0.317	0.626; 0.012	10.1 (1.3)	10.7 (0.5)	10.5 (0.9)	10.9 (0.8)
G_{Tot} (mL min^{-1}/W)	0.061; 0.292	0.637; 0.024	0.954; 0.000	11.5 (1.5)	11.6 (0.6)	12.3 (1.5)	12.4 (1.5)

*The table reports also the mean of the 2.5% and 97.5% percentiles of the 95% confidence interval for τ_2 and the P-values of the ANOVA analysis together with the values of the corresponding partial squared correlation factors. For the meaning of the symbols, please refer to the text. *Significantly different from Pre HIT; †significantly different from Post HIT; ‡significantly different from Pre IRT.*

DISCUSSION

We investigated the effects of *HIT* and *IRT* on $\dot{V}O_2$ kinetics during *CWR HiEx* exercise performed at the same absolute *WR* before and after training in a group of healthy, moderately active elderly men.

Post-intervention assessment after 8 weeks of *HIT* mainly showed:

(i) an increase in $\dot{V}O_{2\,max}$ and an improvement of *RCP;*
(ii) an increase in *Vol* and *CSA* of the quadriceps without a parallel increment of muscular strength;
(iii) a decrease in the amplitude A'_3 of the slow component of $\dot{V}O_2$ kinetics assessed during *HiEx* together with a prolonged d_3;

Post-intervention assessment after 8 weeks of *IRT* showed:

(i) a decrease in the functional gain of the primary phase of $\dot{V}O_2$ kinetics during *ModEx*;
(ii) an increase in muscular *Vol* and *CSA* in parallel with a significant increment in muscular strength;
(iii) a deceleration in the primary component of $\dot{V}O_2$ kinetics with an increased τ_2 during *HiEx*;
(iv) a significant increase of d_3 of $\dot{V}O_{2sc}$ In addition, the longer τ_3 after *IRT* made the kinetics of $\dot{V}O_{2sc}$ significantly slower than the one after *HIT*.

Maximal Oxygen Uptake and Gas Exchange Thresholds

Several studies have demonstrated the efficacy of *HIT* in increasing $\dot{V}O_{2\,max}$ in different populations (Kohrt et al., 1991; Bruseghini et al., 2015). Our results are in line with the findings that 8–12 weeks of interval training can induce a significant increase in $\dot{V}O_{2\,max}$ in elderly subjects (Lepretre et al., 2009). *HIT* induced also a significant improvement in *RCP*. A comparable trend of the positive effects of *HIT* was found at intensities corresponding to the ventilatory threshold in elderly subjects (Pogliaghi et al., 2006).

Muscle Morphology and Strength

The increases of *CSA* (plus 4.3% ± 3.6) and *Vol* (plus 5.6% ± 3.6) found after *HIT* are in agreement with previous findings (Sillanpää et al., 2008; Harber et al., 2012) that showed a significant increase (+6%) of the quadriceps muscle volume in elderly men after 12 weeks of aerobic training paralleled by the increase of *CSA* of myosin heavy chain type I (MHCI) fibers and a higher muscular thickness of *vastus lateralis* and *intermedius* after endurance training in older adults. *IRT* was followed by significant increases of *CSA* and *Vol* (plus 4.2% ± 4.4 and plus 4.9% ± 7.0). This confirms the results obtained in untrained elderly subjects in other occasions (Lee et al., 2008; Shunkert et al., 2008).

IRT was paralleled by a significant increase in the torque produced by the limb extensors. Therefore, we may also reasonably assume that, at *Post IRT*, our subjects were able to pedal at the same *WR* recruiting a smaller number of MUs. Indeed, from the individual *WR* and the isokinetic torque values we can calculate that the average torque maintained by the subject during *HiEx* exercise, when expressed as a percent of T_k, significantly decreased from *Pre IRT* to *Post IRT*: $P = 0.008$, *CI* of the difference 1.3/−0.5 N m for T_k 60° s^{-1}; $P = 0.012$, *CI* of the difference 1.3/−0.25 N m for T_k 120° s^{-1}.

TABLE 2 | Mean values (SD) of the parameters describing [*HHb*] kinetics at the onset of *CWR* exercise of heavy intensity (*HiEx*) together with the values of [*HHb*] and [Hb_{tot}] after 120 s of exercise and at the end of exercise.

Parameter				Training			
				HIT		IRT	
	P_b; η^2_b	P_w; η^2_w	P_{int}; η^2_{inter}	Pre	Post	Pre	Post
$[Hb_{tot}]_{120}$ (μM)	0.052; 0.300	0.814; 0.005	0.772; 0.008	4.5 (3.4)	3.8 (5.6)	6.8 (6.3)	7.0 (6.0)
$[Hb_{tot}]_{end}$ (μM)	0.016; 0.425	0.888; 0.002	0.917; 0.504	4.3 (2.3)	4.3 (6.0)	9.5* (7.5)	9.1 (6.5)
$[HHb]_{A1}$ (s)	0.051; 0.307	0.246; 0.122	0.995; 0.000	8.3 (4.9)	9.8 (6.8)	12.2 (8.0)	13.7 (9.9)
$[HHb]_{d1}$ (s)	0.572; 0.029	0.966; 0.000	0.199; 0.144	5.9 (1.0)	6.7 (3.2)	6.4 (1.4)	5.7 (2.6)
$[HHb]_{\tau\ 1}$ (s)	0.132; 0.188	0.731; 0.011	0.324; 0.091	4.5 (1.1)	4.4 (0.9)	3.9 (1.2)	4.3 (1.0)
95% *IC* $[HHb]_{\tau\ 1}$ (s)				3.1–5.6	3.2–5.8	2.2–6.0	3.6–5.1
$[HHb]_{MRT}$ (s)	0.135; 0.191	0.821; 0.005	0.439; 0.055	10.4 (1.3)	11.1 (3.2)	10.3 (1.9)	10.0 (3.3)
$[HHb]_{120}$ (μM)	0.091; 0.237	0.208; 0.140	0.627; 0.22	5.4 (3.2)	7.3 (6.3)	7.5 (4.6)	8.5 (5.9)
$[HHb]_{end}$ (μM)	0.042; 0.324	0.121; 0.204	0.590; 0.027	5.4 (3.0)	6.4 (4.9)	6.9 (4.1)	9.0 (6.1)
$\Delta[HHb]/\Delta\dot{V}O_2$ (μM L^{-1} min^{-1})	0.078; 0.255	0.425; 0.060	0.922; 0.001	5.6 (3.1)	6.2 (3.7)	8.0 (5.3)	8.5 (6.2)

*$\Delta[HHb]/\Delta\dot{V}O_2$ ratio is also reported. The table reports also the mean of the 2.5% and 97.5% percentiles of the 95% confidence interval for τ_1 and the P-values of the ANOVA analysis together with the values of the corresponding partial squared correlation factors. For the meaning of the symbols, please refer to the text. *Significantly different from Pre HIT.*

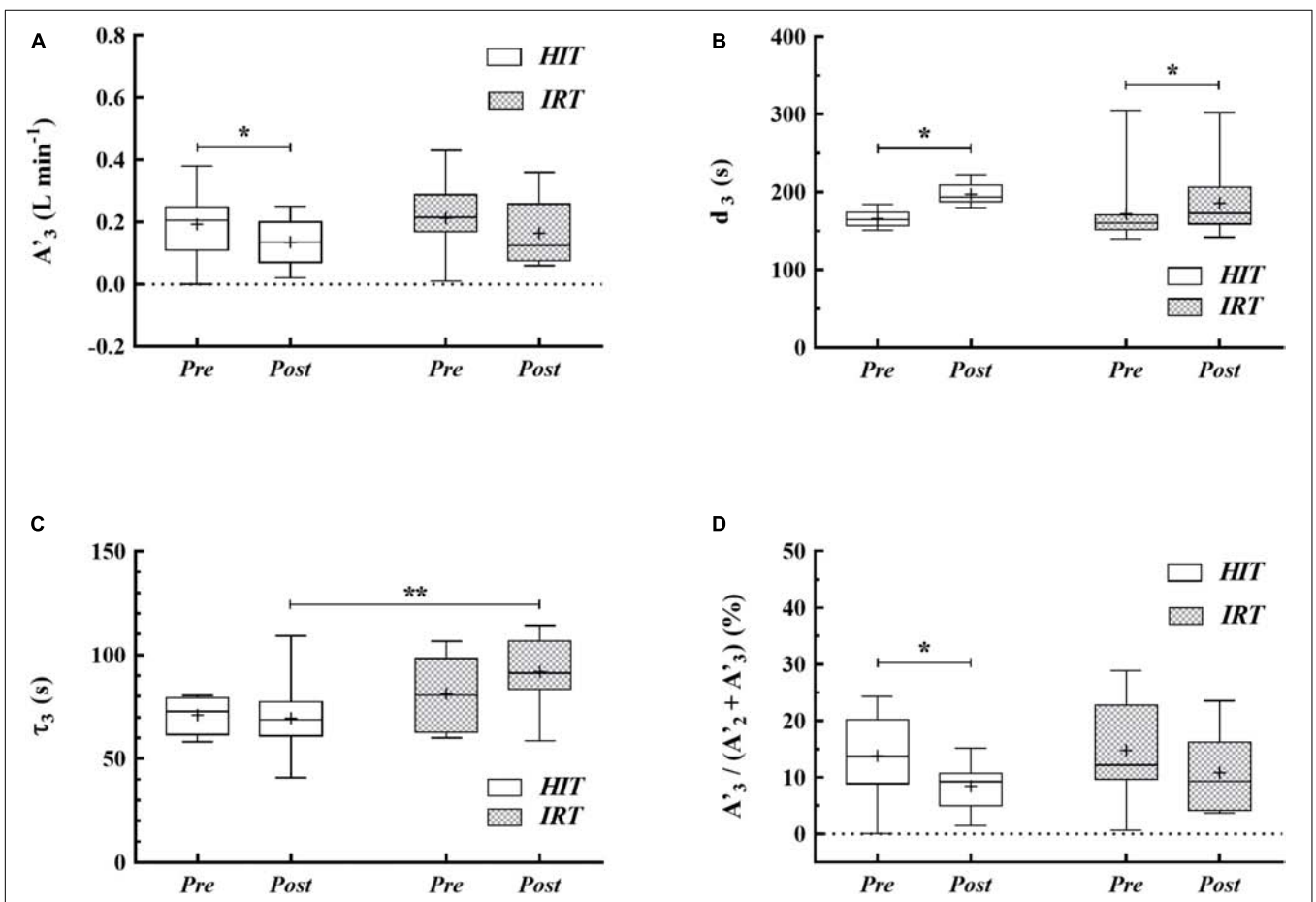

FIGURE 3 | Box-whiskers graphs of the amplitude (A'_3) of the O_2 uptake at the end of exercise **(A)**, time delay **(B)** and slow phase time constant **(C)** of the $\dot{V}O_{2sc}$ and relative contribution of A'_3 to the overall $\dot{V}O_2$ response **(D)** at *Pre HIT*, *Post HIT*, *Pre IRT,* and *Post IRT*. Horizontal line marks the median of the data distribution, box extends from the 25th to the 75th percentiles; whiskers extend down to the 5th percentile and up to the 95th percentile; the cross represents the average. *$P < 0.05$; **$P < 0.01$.

Response to CWR of Moderate Intensity Exercise

The significant decrease in *G* after *IRT* reflected a decrease in the O_2 cost of exercise and translated into a small, albeit significant, increase of 5% in work efficiency, η (*Pre IRT* η 22.9% vs. *Post IRT* η 24.0%; $P = 0.041$; 95% CI_{Diff}: 0.1%/2.0%). This is consistent with previous findings of a significant decrease in the amplitude of the primary phase in cycling (Zoladz et al., 2012) after strength training. Accordingly, strength training results in an improved delta η (Bastiaans et al., 2001) and work η (Sunde et al., 2010) in cycling. The improvement in η found after *IRT* remains difficult to explain, though. One may surmise that, by increasing the absolute strength of the muscles involved in cycling, the subjects were pedaling against the same workload recruiting a smaller number of less efficient Type II fibers, wherefrom a smaller *G* and a larger η derived.

Response to CWR During Heavy-Intensity Exercise

Endurance training is followed by a substantial reduction of $\dot{V}O_{2sc}$ (Casaburi et al., 1987; Womack et al., 1995). In addition, the contribution of $\dot{V}O_{2sc}$ to the overall $\dot{V}O_2$ response has been related to the % of Type I fibers, which have been described to have a greater metabolic stability (Hochacka and McClelland, 1997). Therefore, an increase of the % of Type I fibers at *Post HIT* may have led to improved metabolic stability (Zoladz et al., 2006), attenuated the drop in intramuscular *pH* and *[PCr]* and decreased $\dot{V}O_{2sc}$ (Jones et al., 2007).

In addition to these mechanisms directly linked to the plausible phenotypical shift in muscle fiber populations, also mechanisms intrinsic to each single fiber may be responsible for the observed decrease of $\dot{V}O_{2sc}$ after *HIT*. A recent study (Zoladz et al., 2016) showed that endurance training in rats induced a temperature dependent enhancement of mitochondrial oxidative phosphorylation and a significant drop of mitochondrial uncoupling. Therefore, the decrease of O_2 cost for oxidative *ATP* production in each recruited muscle fiber may have substantially potentiated the effect of endurance training on $\dot{V}O_{2sc}$.

It has been also shown that $\dot{V}O_{2sc}$ is modulated by manipulations of O_2 delivery (Poole and Jones, 2012). *HIT* may improve O_2 availability and induce a better matching between O_2 delivery and utilization (Murias et al., 2010a,b). This may have a positive impact on $\dot{V}O_{2sc}$ in the elderly in whom metabolic vasodilatation is impaired (Poole et al., 2003) and a mismatch of local O_2 delivery to O_2 muscular consumption is present (Murias et al., 2010a,b). However, the obtained results do not support this conclusion, as training did not modify any of the indexes that characterize *HHb* response during *HiEx* exercise. In particular, the primary time constant $[HHb]_{\tau\ 1}$ was not affected: a constant τ_1 would suggest a proportionally similar increase of the speeds of adjustment of local O_2 delivery and muscular O_2 uptake in the primary phase of $\dot{V}O_2$ kinetics during *HiEx*. This conclusion is somehow strengthened by the observation that $[HHb]_{end}$ was not modified in presence of a lower A'_3 of O_2 uptake response.

The primary phase of τ_2 of $\dot{V}O_2$ kinetics during *HiEx* was decelerated after *IRT*. In analogy with *ModEx*, the primary component in *HiEx* exercise is thought to increase exponentially without other changes (Poole and Jones, 2012). However, because the statistical estimate of τ_2 during *HiEx* is often based on a limited number of data, this unavoidable drawback may produce uncertain and unreliable values of τ_2. Besides these methodological problems, specific physiological adaptations induced by *IRT* may have contributed to the increase of τ_2. A constant $[HHb]_\tau$ helps us infer that local O_2 delivery response and muscular O_2 utilization changed proportionally after training. Therefore, a substantial defect in O_2 availability may be still present after *IRT*.

The changes induced by *IRT* on $\dot{V}O_{2sc}$ are somehow ambiguous: the amplitude of $\dot{V}O_{2sc}$, either in absolute or relative terms, turned out to be unaffected by *IRT*, but $\dot{V}O_{2sc}$ appeared later and developed more slowly than at *Post HIT*.

The mechanism underpinning the delay in the appearance of $\dot{V}O_{2sc}$ after *HIT* and *IRT* are of different origin. We first underline that *IRT* training was effective in increasing the strength of muscles involved in pedaling (see the section "Results"). Therefore, we can suggest that the pedaling subjects after *IRT* were utilizing a lower percentage of their maximal voluntary force at the same *WR* and that they were recruiting a smaller number of Type II MUs. Should this be true, the diminished recruitment of these MUs would result in a slower development of $\dot{V}O_{2sc}$, as the utilized Type I muscle fibers are less liable to develop fatigue and their metabolic features make them less prone to cause $\dot{V}O_{2sc}$. However, this explanation does not clarify whether the main cause of $\dot{V}O_{2sc}$ resides in *intensive* mechanisms, i.e., the progressive decay of the efficiency of the already recruited MUs, or, rather, it may be ascribed to an *extensive* process, i.e., the progressive recruitment of less efficient Type II fibers. It is worth noting, however, that the net decrease of d_3 observed after *IRT* was positively correlated with the net increase of *knee torque* ($P = 0.046$, $r = 0.60$). Conversely, the two variables were not correlated in the case of *HIT* ($P = 0.316$, $r = -0.32$).

Also, after IRT, we were not able to find any significant changes of muscular oxygenation and of the indexes that describe amelioration of local peripheral perfusion. This might suggest that the impairment of local O_2 delivery was not the main cause of $\dot{V}O_{2sc}$, at least in this specific population of subjects.

Points of Strength and Weakness of the Study

We compared for the first time the effects of *HIT* and of *IRT* on the dynamic response of pulmonary $\dot{V}O_2$ and muscular oxygenation during *HiEx* exercise in healthy, untrained elderly men.

However, a few methodological limitations should be mentioned. The experimental design was not counterbalanced for reasons of feasibility.

We did not evaluate the changes in muscle fiber expression during the two training interventions. Since the size of $\dot{V}O_{2sc}$ has been positively related to the percentage of Type II

fibers (Poole and Jones, 2012), a strong correlation between the observed changes in the slow component and the changes in the phenotypical expression of the trained muscles would have strengthened the hypothesis of a muscular origin of the slow component.

We did not evaluate the possible changes in neuromuscular activation induced by the two training modalities during *ModEx* and *HiEx*. Comparison of the differences in recruitment patterns would have helped to strengthen or reject our working hypothesis on the role of Type II motor units involvement in the genesis of $\dot{V}O_{2sc}$ after *IRT*.

HHb signal mainly reflects the fractional O_2 extraction of the interrogated zone of the muscle resulting from the dynamic balance between muscular O_2 uptake and local O_2 delivery and *HHb* signal reflects changes in oxygenation mainly in the capillaries of the explored muscle volume (Grassi and Quaresima, 2016). However, the assessment of *HHb* obtained only from the surface of the *vastus lateralis* may be a substantial limitation to our analysis, since some spatial heterogeneity in terms of muscle oxygenation in an exercising muscle seems to exist (Koga et al., 2007). Nonetheless, using skin landmarks to accurately place the NIRS probe in the same site before all experiments minimized possible problems due to spatial inhomogeneity.

Finally, the study aimed to investigate the effects of training in a particular population of subjects, i.e., elderly healthy volunteers who may have larger strength deficits than young, active adults. Therefore, the meaning and the applicability of the results obtained in this study may be extended with some caution to other classes of subjects.

CONCLUSION

The amplitude of $\dot{V}O_{2sc}$ during *HiEx* was substantially smaller after *HIT* than before, but its decrease was not correlated with an improvement in the O_2 delivery-to-utilization ratio of the exercising muscles. This suggests that suboptimal local O_2 delivery was not a possible factor contributing to $\dot{V}O_{2sc}$ in the elderly, whereas the improved metabolic stability induced by *HIT* was likely able to induce beneficial effect on $\dot{V}O_{2sc}$.

IRT, by increasing muscle strength, resulted in a delayed appearance of $\dot{V}O_{2sc}$ during *HiEx* because of a possible larger contribution of Type I fibers to a motor task of identical absolute intensity.

Perspectives

The results obtained in the present investigation may have practical applications. First, the association between a larger exercise economy and the delayed appearance of $\dot{V}O_{2sc}$ found after *IRT* may be of interest, as it suggests that strength training should be included in the usual training programs of elderly people to improve exercise economy and resistance to fatigue (Beattie et al., 2014). Second, they prompt the investigators to better characterize the changes in neuromuscular activation and MUs recruitment induced by the two training modalities during *HiEx* performed at the same *WR*. This should be done in parallel with the invasive evaluation of the changes in muscle fiber expression induced by training interventions.

AUTHOR CONTRIBUTIONS

CC, PB, ET, FS, and EC planned the study. CC, ET, PB, EO, AP, RPM, SP, and EC collected and analyzed the data. CC, PB, and ET wrote the manuscript. CC, PB, ET, EC, and RPM revised the manuscript.

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Functional Connectivity Within the Executive Control Network Mediates the Effects of Long-Term Tai Chi Exercise on Elders' Emotion Regulation

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Previous research has identified the effects of tai chi exercise on elders' executive control or on their emotion regulation. However, few works have attempted to reveal the relationships between tai chi, executive control, and emotion regulation in the same study. The current resting-state study investigated whether the impact of tai chi on elders' emotion regulation was mediated by the resting-state functional connectivity within the executive control network. A total of 26 elders with long-term tai chi experience and 26 demographically matched healthy elders were recruited. After the resting-state scan, both groups were required to complete a series of questionnaires, including the Five Facets Mindfulness Questionnaire (FFMQ), and a sequential decision task, which offered an index of the subjects' emotion-regulation ability by calculating how their emotional response could be affected by the objective outcomes of their decisions. Compared to the control group, the tai chi group showed higher levels of non-judgment of inner experiences (a component of the FFMQ), stronger emotion-regulation ability, and a weaker resting-state functional connectivity between the dorsolateral prefrontal cortex (DLPFC) and the middle frontal gyrus (MFG). Moreover, the functional connectivity between the DLPFC and the MFG in the tai chi group fully mediated the impact of non-judgment of inner experience on their emotion-regulation ability. These findings highlighted that the modulation of non-judgment of inner experience on long-term tai chi practitioners' emotion regulation was achieved through decreased functional connectivity within the executive control network.

Keywords: tai chi, meditation, emotion regulation, executive control network, resting-state functional connectivity, functional magnetic resonance imaging

INTRODUCTION

Emotional suffering in older adults decreases their quality of life and increases their risk for mental disease, such as depression and anxiety (Berkman et al., 1986; Rozzini et al., 2001). A growing body of research has demonstrated that emotional suffering in older adults is associated with age-related cognitive impairment (Lesser et al., 1996; Butters et al., 2004), which is reflected by changes in a series of indicators such as gray matter volume, brain activity during tasks, and brain functional connectivity in the resting state. The pathology of brain networks in cognitive impairment in older adults has been investigated by applying resting-state functional connectivity (Liu et al., 2015; Liu F. et al., 2017; Lin et al., 2018). Most of these studies revealed that a key brain network in cognitive impairment was the executive control network, which influences many domains of life, such as academic success and healthy eating (Robinson et al., 2010; Hofmann et al., 2012). The prevention of declining executive control appears to be important and should become one of the best long-term strategies to achieve successful aging.

The organization and function of the human brain throughout life is plastic (Kurth et al., 2015). Evidence regarding the plasticity of executive control function can be demonstrated by mind-body training (Tang et al., 2015). The most well-researched mind-body training for executive control function is meditation (Teper and Inzlicht, 2013), which is thought to be able to promote executive control function through improving present-moment awareness and non-judgmental acceptance, thus achieving increased sensitivity to affective cues that help signal the need for control (Cardaciotto et al., 2008; Teper et al., 2013). Thus, it is not surprising that there is a wealth of evidence to support the association between meditation training and improved executive control function on both behavioral and neural levels (Hölzel et al., 2011; Zanesco et al., 2013). For example, Wenksormaz (2005) found that engaging in meditation training improved executive control function. Tao et al. (2017) recently revealed that meditation training enhanced executive control function by decreasing the resting-state functional connectivity between the key regions such as the dorsolateral prefrontal cortex (DLPFC) and the frontal regions in the central executive control network. Researchers found that compared to the meditation training group, older adults in the control group might compensate for disruption of the executive control network by recruiting additional frontal resources to overcome executive control deficits (Gutchess et al., 2007; Tao et al., 2017). Therefore, meditation training can reduce compensation for executive control function, which was reflected by weaker functional connectivity within the executive control network among meditators. Moreover, meditation was often described as non-judgmental acceptance or regulation of the present emotional experiences (Teper et al., 2013). Improvements in emotion regulation associated with meditation have been investigated through self-reporting and physiological and neuroimaging methods (Fox et al., 2012; Tang and Posner, 2012). For instance, Ortner et al. (2007) found that meditators represented reduced emotional responsiveness to unpleasant situations, suggesting an enhancement in emotion regulation to avoid the potentially harmful effects of negative emotions. Taken together, prior studies have shown the remarkable influence of meditation on both executive control function and emotion regulation. However, these previous studies failed to provide direct evidence for the relationship among meditation, executive control, and emotion regulation. The current report aimed to explore this issue.

Meditation encompasses a family of complex practices that include mindfulness meditation, yoga, and tai chi (Tang et al., 2015). Of these, tai chi, a multimodal mind-body exercise that incorporates physical, cognitive, and meditative components in the same activity is growing in popularity, especially among older adults (Wayne et al., 2014). Previous studies have suggested that tai chi, as a physical exercise, was an effective method not only to improve health fitness, such as neuromuscular functions and cardiorespiratory system and balance control (Ray et al., 2005; Wang et al., 2010; Ghaffari and Kluger, 2014), but also benefit emotion regulation and psychological well-being in elders (Wang et al., 2010). The current resting-state functional magnetic resonance imaging (fMRI) study (containing a tai chi group and a control group) aimed to replicate previous findings that meditative components in tai chi were related to enhanced executive control and stronger emotion regulation. More importantly, taking the functional connectivity of the executive control network in the resting state as an indicator of executive control, we tried to assess whether tai chi achieves inducing a stronger emotion-regulation ability via enhancing the function of the executive control network.

The main focus of the current study was to examine whether the impact of the meditative component of tai chi on emotion regulation was mediated by the functional connectivity between the DLPFC (a core region of the executive control network) (Sheline et al., 2010) and the frontal regions. Behaviorally, we predicted that the tai chi group would have a higher meditation score and stronger emotion regulation than the control group. At the neural level, in line with previous findings, we predicted that the tai chi group would show weaker functional connectivity between the DLPFC and the frontal regions, such as the middle frontal gyrus (MFG) in the resting state. Finally, we predicted that the impact of meditation on emotion regulation in the tai chi group was mediated by functional connectivity between the DLPFC and the MFG.

MATERIALS AND METHODS

Subjects

Totally 26 tai chi practitioners and 26 control subjects were recruited from the community. The subjects in the tai chi group had engaged in tai chi for an average of 10.44 ± 5.48 years. The control participants were active in other types of physical exercise without a meditation component, such as jogging and square dancing. The subjects' demographic characteristics are provided in **Table 1**. All of the subjects (1) did not have any neurological diseases, history of stroke, or severe cerebrovascular diseases, (2) had normal or corrected-to-normal vision, (3) had the ability to provide written informed consent, and (4) were right-handed.

TABLE 1 | Demographics of the tai chi and control groups.

Characteristics	Age (years)	Gender (male/female)	Education (years)	Tai chi (years)	Exercise time per day (min)
Tai chi group	65.19 ± 2.30	8/18	10.46 ± 1.79	10.44 ± 5.48	66.76 ± 20.51 (Tai chi)
Control group	63.92 ± 2.87	9/16	11.04 ± 2.57	0	61.54 ± 25.62 (Other exercise)
t	1.751	0.157	−0.934	NA	0.804
p	0.086	0.692	0.355	NA	0.425

All of the subjects provided written informed consent before the study began. One control subject was excluded due to severe head motion (>2 mm or 2°). The remaining 26 tai chi subjects and 25 control subjects were included in the data analyses. This study was approved by the Ethics Committee on Human Experiments of East China Normal University. The protocol was approved by the Ethics Committee on Human Experiments of East China Normal University. All subjects gave written informed consent in accordance with the Declaration of Helsinki.

Procedures

Questionnaires

Before scanning, the subjects were required to complete the Chinese version of the Beck Depression Inventory (BDI) (Beck et al., 1987; Kurylo and Stevenson, 1992), the NEO Five-Factor Inventory (NEO-FFI) (Kurylo and Stevenson, 1992), the Five Facets Mindfulness Questionnaire (FFMQ) (Baer et al., 2006), and the Mindful Attention Awareness Scale (MAAS) (Brown and Ryan, 2003).

Beck Depression Inventory

The BDI was used to assess the depression level of participants. The BDI included 21 items, which was used to measure the symptoms associated with depression. The split-half coefficient of the Chinese version of the BDI was 0.879 and Cronbach's alpha was 0.890. The BDI and its individual items were shown to have good construct and concurrent validities in China (Zhang, 1990).

NEO Five-Factor Inventory

The personality of participants was measured by using the NEO-FFI, a questionnaire addressing five core personality traits: neuroticism, extraversion, openness, conscientiousness, and agreeableness. Each dimension consisted of 12 statements. Participants were asked to rate the degree to which they agree with these statements. Each statement was rated on a 5-point scale (1 = completely agree, 5 = completely disagree), yielding a scale score ranging from 12 to 60.

Five Facets Mindfulness Questionnaire

The FFMQ was used to assess the meditation level of the participants. The FFMQ consisted of 39 items that were rated on a 5-point Likert-type scale (1 = never or very rarely true, 5 = very often or always true). This scale measures five distinct facets of mindfulness: (1) observing (defined in terms of noticing or attending to internal and external experiences, e.g., I notice the smells and aromas of things), (2) describing (defined in terms of labeling internal experiences with words, e.g., I am good at finding words to describe my feelings), (3) acting with awareness (defined in terms of attending to one's activities of the moment, e.g., reverse-scoring item: I am easily distracted), (4) non-judgmental of inner experience (defined in terms of taking a non-evaluative stance toward thoughts and feelings, e.g., reverse scoring item: I disapprove of myself when I have irrational ideas), and (5) non-reactivity to inner experience (defined in terms of allowing thoughts and feelings to come and go, without getting caught up in or carried away by them, e.g., I watch my feelings without getting lost in them). Baer et al. (2006) concluded that the FFMQ had an adequate-to-good internal consistency with the following Cronbach coefficients: observing = 0.83, describing = 0.91, acting with awareness = 0.87, non-judgmental of inner experience = 0.87, and non-reactivity to inner experience = 0.75. The Chinese version of the FFMQ had acceptable psychometric properties and was a valid instrument for the assessment of mindfulness (Deng et al., 2011).

Mindful Attention Awareness Scale

The MAAS was a 15-item instrument measuring the general tendency to be attentive to and aware of present-moment experience in daily life. It had a single factor structure and yielded a single total score. Using a 6-point Likert-type scale (almost always to almost never), respondents rate how often they have experiences of acting on automatic pilot, being preoccupied, and not paying attention to the present moment. Brown and Ryan (2003) reported an internal consistency (coefficient alpha) of 0.82 and expected convergent and discriminant validity correlations. The MAAS and its individual items were shown to have good construct and concurrent validities in China (Deng et al., 2012).

Tasks

The results of the resting-state scans used in the current study were collected immediately after the anatomical scan. The duration of the resting-state scan was 8 min and 6 s. The subjects were asked to stay awake and remain motionless with their eyes closed and ears plugged during the resting-sate scan.

After the resting-state scan, the subjects were instructed to undertake a sequential decision task (see the experimental processes in Brassen et al., 2012). The sequential decision task was proven to be solid by previous studies (Brassen et al., 2012; Liu et al., 2016; Liu F. et al., 2017; Li et al., 2018). By using this task, we acquired information on the subjects' emotion changes to different outcomes. The subjects were informed that they would obtain tokens (gold coins) from the task and that payment for their participation was determined by the total number of tokens they obtained (1 token for 1 Chinese yuan).

All of the subjects participated in 80 trials of the sequential decision task. For each trial, eight boxes were presented, seven containing gold coins and one containing a devil. The position

of the devil was set randomly at each trial. The subjects were instructed to open the boxes from left to right and stop when they wanted to collect the coins acquired thus far. They had to decide whether to open the next box or collect their coins within 2 s by pressing a button. Exposing the randomly distributed devil ended the trial, and all of the tokens from the trial were lost. If the subjects stopped and collected their gains, the position of the devil was revealed, thus informing the subjects about both the number of gold coins they gained and the number they had missed. The outcome of each trial was one of the following two conditions: (1) a gain condition, in which the subjects did not unpack the devil and gained gold coins in that trial and (2) a loss condition, in which the subjects unpacked the devil and lost the gold coins collected in that trial. A jittered interval was presented either after the subjects decided to stop or after they unpacked the devil. Next, the outcome was presented for 3 s. This was highlighted on a screen by a cyan square (in the case of stopping and collecting the gains) or by a red square (in the case of unpacking the devil and losing the gains in that trial). Finally, the emotional rating stage was presented. At this stage, the subjects were asked to rate how they felt about their choices on a 9-point scale from extreme regret (defined as −4) to extreme relief (defined as 4) in 3 s.

fMRI Data Acquisition

The fMRI data were acquired using a 3.0-T Siemens Trio system scanner (East China Normal University, Shanghai, China). Prior to the resting-state stage, a high-resolution structural image was acquired using a T1-weighted, multiplanar reconstruction (MPR) sequence [repetition time (TR) = 1900 ms, echo time (TE) = 3.42 ms, 192 slices, slice thickness = 1 mm, field of view (FOV) = 256 mm, matrix size = 256 × 256]. Resting-state fMRI data were acquired using a gradient-echo echo-planar imaging (EPI) sequence (TR = 2000 ms, TE = 30 ms, FOV = 220 mm, matrix size = 64 × 64, 35 slices, slice thickness = 4 mm).

Data Analyses

Demographic Characteristics and Scale Data Analyses

To investigate the different demographic characteristics and scale scores between the two groups, independent samples *t*-tests and Chi-squared tests were conducted using SPSS 18.0 software. A threshold of $p < 0.05$ (two-tailed) was applied.

Behavioral Data Analyses

In the gain condition, we calculated a combined index, called the real gain percentage (RGP), which was defined as the ratio of the collected gains and the largest possible gains (that is, the total number of boxes before the devil) in a given trial (Liu et al., 2016; Liu Z. et al., 2017; Li et al., 2018). The RGP value can be considered an indication of how good the outcome is in a particular trial. Regression analyses were performed for each subject to investigate the differences in the subjects' sensitivity to the objective outcomes between the two groups. The RGP value was defined as a predictor and emotional ratings as independent variables (see the following equation).

$$\text{Emotional rating} = \text{K} \times \text{RGP value} + \text{b}$$

Each subject's regression coefficient (K) and intercept (b) were calculated. K was considered an index of their sensitivity to the objective outcomes in the gain condition. Independent samples *t*-tests in SPSS 18.0 software were performed to investigate the differences between the two groups in emotional stability. A threshold of $p < 0.05$ (two-tailed) was applied.

In the loss condition, we also performed regression analyses for each subject in which the number of lost coins was defined as a predictor and the emotional ratings as independent variables. Independent samples *t*-tests in SPSS 18.0 software were performed to compare the differences in regression coefficients of the two groups. A threshold of $p < 0.05$ (two-tailed) was applied.

fMRI Data Preprocessing

Data preprocessing was performed using the Data Processing Assistant for Resting-State fMRI Advanced (DPARSFA[1]) in MATLAB. The DPARSFA software was based on Statistical Parametric Mapping (SPM8[2]) and the Resting-State fMRI Data Analysis Toolkit (REST[3]). The first 10 volumes were not analyzed to allow for the signal equilibration of each subject. The remaining 230 time points from each subject were corrected for the delay in slice acquisition. Afterward, the images were realigned and head motions corrected and coregistered to the respective T1-weighted structural images of each subject. The coregistered structural images were then segmented into gray matter (GM), white matter (WM), and cerebrospinal fluid (CSF) using a unified segmentation algorithm. Next, the 6 rigid body motion parameters, WM, and CSF signals were regressed out. The functional images were spatially normalized to the Montreal Neurological Institute (MNI) space (resampled to 2 mm × 2 mm × 2 mm) using the normalization parameters estimated during unified segmentation and spatially smoothed with a Gaussian kernel of 6-mm full-width half-maximum (FWHM) and linearly detrended. Finally, the data were bandpass filtered from 0.01 to 0.08 Hz.

fMRI Data Analyses

Given the importance of the DLPFC to executive function, we chose the DLPFC (seed region, MNI 36 27 29) as the region of interest based on previous research that demonstrated that the DLPFC was a vital region in the CCN network (Fales et al., 2008; Sheline et al., 2010; Hwang et al., 2015). For each subject, seed-based FC was computed as the Pearson correlation coefficients between the seed region (a 6-mm sphere around the coordinates of the DLPFC) and other voxels of the whole brain. The correlation coefficients were then z-transformed for standard purposes (the fisher r to z), and seed-based FC maps were generated. Group differences were compared using voxel-wise independent sample *t*-tests. Moreover, to explore the association between the resting-state functional connectivity and behavioral variables (meditation score and *K*-value), we also performed regression analyses. For all of the fMRI analyses, age, gender, and years of education were included in the analysis as covariates

[1]http://rfmri.org/DPARSF

[2]http://www.fil.ion.ucl.ac.uk/spm

[3]http://www.restfmri.net

of non-interest. Finally, a cluster-level threshold of $p < 0.05$ (familywise error, FWE) and a voxel-level threshold of $p < 0.005$ (uncorrected) were used to define activations.

RESULTS

Demographic Data and Scale Data

Two groups showed no significant differences in demographics, such as age [$t_{(49)} = 1.751$, $p = 0.086$], gender [$t_{(49)} = 0.157$, $p = 0.692$], education [$t_{(49)} = -0.934$, $p = 0.355$], and physical exercise time per day [$t_{(49)} = 0.804$, $p = 0.425$] (**Table 1**). The tai chi group scored higher on non-judgment of inner experience [$t_{(49)} = 2.336$, $p < 0.05$], non-reactivity [$t_{(49)} = 3.097$, $p < 0.01$], total FFMQ scores [$t_{(49)} = 2.277$, $p < 0.05$], and MAAS scores [$t_{(49)} = 2.447$, $p < 0.05$] relative to the control group. No other significant difference in scales was found between the two groups (**Table 2**).

Behavioral Results

In the gain condition, the relationships between the subjects' emotional ratings and RGP in both the tai chi and control groups were described (**Figure 1A**). Moreover, independent samples *t*-tests showed that the *K*-value in the tai chi group was significantly smaller than in the control group [$t_{(49)} = 4.82$, $p < 0.01$] (**Figure 1B**). This result indicated that the tai chi group showed less sensitivity to the objective outcomes than the control group. In addition, the result showed non-judgment of inner experience was negatively correlated with the *K*-value across all subjects ($r = -0.365$, $p < 0.05$). Further analysis found the significant correlation between non-judgment of inner experience and *K*-value in the tai chi group ($r = -0.481$, $p < 0.05$). We did not find such a correlation in the control group ($r = 0.057$, $p > 0.05$) (**Figure 2**).

In the loss condition, the regression coefficient in the tai chi group had a tendency to be smaller relative to the control group, although this did not reach statistical significance [$t_{(49)} = 1.757$, $p = 0.085$] (**Figure 1C**).

fMRI Results

Independent Samples *t*-Tests

Seed-based functional connectivity was computed as the Pearson correlation coefficients between the seed region (DLPFC) and other voxels of the whole brain. The results revealed that the subjects in the tai chi group showed significantly decreased resting-state functional connectivity between the DLPFC (seed region) and the left thalamus (MNI −20 −14 6), left ventral striatum (MNI −26 −8 2), and right MFG (MNI 3848 10) compared to the subjects in the control group (**Figure 3** and **Table 3**). The subjects in the tai chi group did not show significantly stronger functional connectivity than those in the control group when the seed region was the DLPFC.

Correlation Analyses

In the tai chi group, non-judgment of inner experience was negatively associated with functional connectivity between the DLPFC and the right MFG (MNI 44 −4 60) (**Figure 4A** and **Table 4**). The functional connectivity strength between the DLPFC and the MFG (MNI 44 −4 60) was then calculated. In order to express the results more clearly, **Figure 4B** reveals that the functional connectivity strength between the DLPFC and the MFG was negatively correlated with non-judgment of inner experience ($r = -0.505$, $p < 0.01$). Moreover, the *K*-value and the resting-state functional connectivity between the DLPFC and the right MFG (MNI 38 −6 54) showed a positive correlation

TABLE 2 | Scale data of the tai chi and control groups.

Psychological measures		Tai chi group	Control group	*t*	*p*
BDI					
	Depressive	4.69 ± 5.36	5.36 ± 4.86	−0.465	0.644
NEO-FFI					
	Neuroticism	27.31 ± 7.62	30.92 ± 6.19	−1.854	0.070
	Extraversion	42.1 ± 6.74	40.76 ± 4.90	0.865	0.391
	Openness	39.77 ± 5.14	39.64 ± 4.64	0.634	0.529
	Agreeableness	45.11 ± 6.57	44.16 ± 5.69	0.554	0.582
	Conscientiousness	48.27 ± 5.65	48.04 ± 5.04	0.153	0.879
FFMQ					
	Observing	3.50 ± 0.79	3.21 ± 0.62	1.427	0.160
	Describing	3.55 ± 0.66	3.48 ± 0.50	0.415	0.680
	Act with awareness	3.68 ± 0.95	3.74 ± 0.70	−0.267	0.791
	Non-judging	2.86 ± 0.49	2.55 ± 0.46	2.336	0.024*
	Non-reactivity	3.58 ± 0.46	3.11 ± 0.60	3.097	0.003**
	Total FFMQ score	17.16 ± 1.83	16.10 ± 1.48	2.277	0.027*
MAAS					
	Total MAAS score	72.54 ± 11.72	63.64 ± 14.18	2.447	0.018*

*BDI, Beck Depression Inventory; NEO-FFI, NEO Five-Factor Inventory; FFMQ, Five Facets Mindfulness Questionnaire; MAAS, Mindful Attention Awareness Scale. *$p < 0.05$, **$p < 0.01$.*

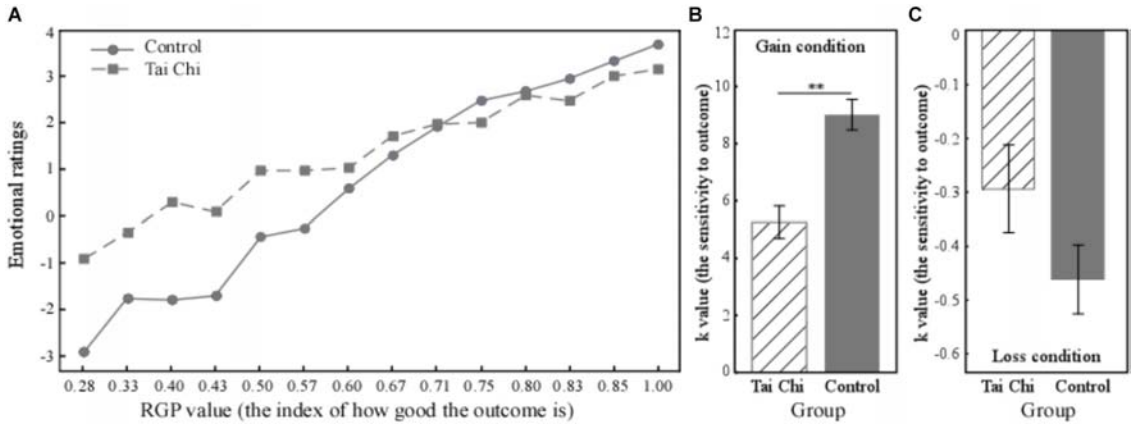

FIGURE 1 | The relationship between the subjects' emotional ratings and RGP in the tai chi group **(A)**. The independent samples *t*-test revealed that the regression coefficient (K) in the tai chi group was significantly smaller than in the control group [$t_{(49)} = 4.82$, $p < 0.01$] **(B)**. The regression coefficient (K) showed no significant difference between two groups in Loss condition [$t(49) = 1.757$, $p = 0.085$] **(C)**. $^{**}p < 0.01$.

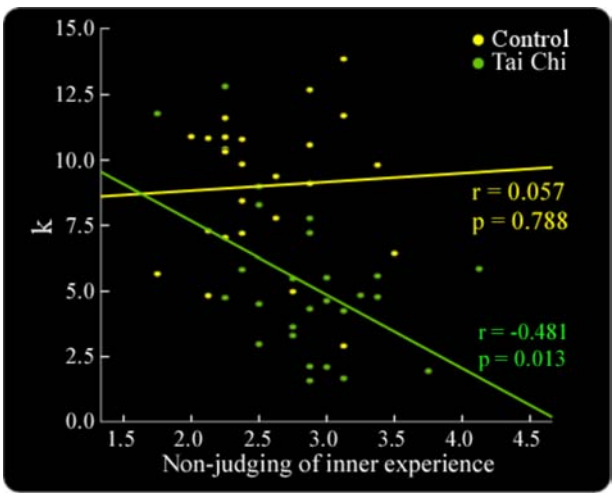

FIGURE 2 | The correlation analyses showed that non-judgment of inner experience was negatively correlated with the *K*-value in the tai chi group ($r = -0.481$, $p < 0.05$). No such correlation was found in the control group ($r = 0.057$, $p > 0.05$).

in the tai chi group (**Figure 5A** and **Table 5**). **Figure 5B** describes the relationship between the *K*-value and the DLPFC resting-state functional connectivity ($r = 0.563$, $p < 0.01$). No aforementioned correlation was found in the control group. In addition, both the functional connectivity strength between the DLPFC and the thalamus and the functional connectivity strength between the DLPFC and the ventral striatum did not show significant correlation with non-judgment of inner experience or the *K*-value.

Mediation Analyses

To test whether the effect of non-judgment of inner experience on the subjects' sensitivity to outcomes (K) was mediated by the functional connectivity between the DLPFC and the MFG, we conducted a mediation analysis. The mediation analysis showed that there was no significant effect of non-judgment of inner experience on the subjects' sensitivity to outcomes after including the functional connectivity between the DLPFC and the MFG (path A: $beta = -0.505$, $p < 0.01$; path B: $beta = 0.563$, $p < 0.01$; path C: $beta = -0.481$, $p < .05$; path c': $beta = -0.265$, $p > 0.05$) (**Figure 6**). Thus, the functional connectivity between the DLPFC and the MFG fully mediated the impact of non-judgment of inner experience on the subjects' sensitivity to outcomes.

DISCUSSION

In the current cross-sectional study, we combined resting-state fMRI and a sequential decision task to investigate whether the impact of the meditative component of tai chi on emotion regulation was mediated by resting-state functional connectivity within the executive control network. Behaviorally, the tai chi group showed a higher propensity to adopt a non-judgmental stance toward their inner experience and less sensitivity to outcomes (the reflection of stronger emotion-regulation ability) than the control group. Furthermore, we found that the non-judgment score of inner experience was negatively correlated with the subjects' sensitivity to outcomes in the tai chi group. At the neural level, the tai chi group showed decreased functional connectivity between the DLPFC and the MFG compared to the control group. In addition, the functional connectivity between the DLPFC and the MFG was negatively correlated with non-judgment of inner experience and positively correlated with the subjects' sensitivity to outcome only in the tai chi group. Interestingly, the functional connectivity between the DLPFC and the MFG fully mediated the impact of non-judgment of inner experience on the tai chi group's sensitivity to outcome.

In line with our hypothesis, the results showed that long-term tai chi exercise was associated with enhanced non-judgment of inner experience and reduced sensitivity to outcomes. In the tai chi group, a strong stance to not judge inner

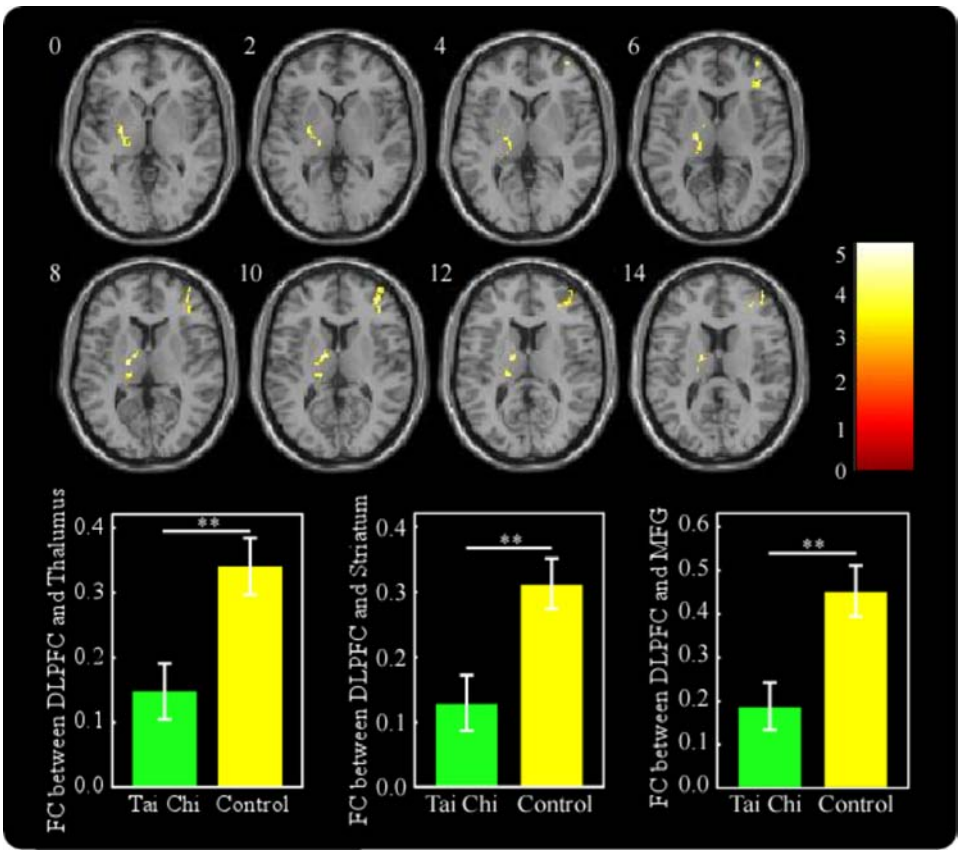

FIGURE 3 | The tai chi group showed significantly decreased resting-state functional connectivity between the DLPFC and the left thalamus (MNI –20 –14 6), left ventral striatum (MNI –26 –8 2), and right MFG (MNI 3848 10) compared to the control group. $^{**}p < 0.01$

TABLE 3 | The difference in resting-state functional connectivity of the tai chi and control groups.

		Peak activation				
Region		***X***	***Y***	***Z***	***t*-Value**	**Voxels**
Seed region: DLPFC						
Tai chi* < *control						
L	Thalamus	−20	−14	6	4.16	165
L	Pallidum	−26	−8	2	3.93	
R	MFG	38	48	10	3.87	123
Tai chi* > *control						
	No region					

Coordinates (mm) are in MNI space. L, left hemisphere; R, right hemisphere. All of the clusters survived FWE correction ($p < 0.05$) for multiple comparisons at the cluster level, with a voxel-level threshold corresponding to $p < 0.005$, uncorrected.

experience might have mitigated the abstract thoughts evaluating the characteristics of outside stimuli, thereby reducing the intensity of emotional sensitivity to outcomes, that is, improving emotional stability. Thus, tai chi training might improve emotional stability in older adults as a reflection of stronger emotion regulation. For those in the tai chi group, non-judgment of inner experience was positively correlated with their emotional stability, supporting the notion that increased emotional stability may be an outcome of long-term meditation training (Tang et al., 2015, 2016). Previous work has shown that meditation was an effective way to improve individuals' core psychological and cognitive abilities, including emotion regulation (Lutz et al., 2008; Moyer et al., 2011). Literature reported that the connection between meditation and improved emotion regulation was certainly intuitive regarding the emphasis on the non-judgmental acceptance of thoughts and emotions at the core of meditation training (KabatZinn, 2014). Long-term tai chi practitioners might learn to intentionally observe and accept affective states and were able to reduce habitual tendencies to ruminate about their feelings (Brown et al., 2013) as well as strengthen adaptive processing of emotional information (Farb et al., 2013). Taken together, our results suggested that long-term tai chi practice is an effective way to improve emotion regulation in older adults, where enhanced non-judgment of inner experience through tai chi training might play an important role.

Mind-body exercises such as tai chi and yoga can significantly enhance cognitive function by modulating the brain functioning and structures associated with cognitive processes (Wei et al., 2015; Afonso et al., 2017). In recent years, the popularity of resting-state functional connectivity has further endorsed this method of investigating the brain as a network (Li et al., 2014;

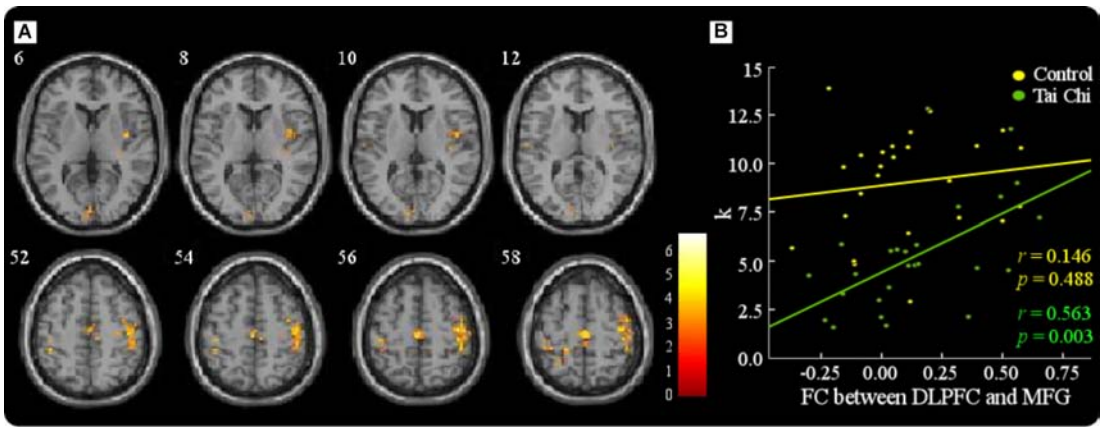

FIGURE 4 | In the tai chi group, non-judgment of inner experience had a negative association with functional connectivity between the DLPFC and the right MFG (MNI 44 –4 60) **(A)**. The results showed that the functional connectivity strength between the DLPFC and the MFG was negatively correlated with non-judgment of inner experience in the tai chi group ($r = –0.505$, $p < 0.01$). No correlation was found in the control group ($r = 0.07$, $p > 0.05$) **(B)**.

TABLE 4 | The correlation between non-judgment of inner experience and resting-state functional connectivity in the tai chi group (seed region: DLPFC).

		Peak activation				
Region		***X***	***Y***	***Z***	***t*-Value**	**Voxels**
Negatively correlated with non-judging of inner experience						
L	Precentral gyrus	−38	−14	58	5.68	245
L	Superior frontal gyrus	−22	−8	74	3.48	
R	Precentral gyrus	64	−12	36	4.63	193
R	Rolandic operculum	58	−8	10	4.73	136
R	Precentral gyrus	42	−16	56	4.3	89
R	MFG	44	−4	60	3.54	
Positively correlated with non-judging of inner experience						
	No region					

Coordinates (mm) are in MNI space. L, left hemisphere; R, right hemisphere. All of the clusters survived FWE correction ($p < 0.05$) for multiple comparisons at the cluster level, with a voxel-level threshold corresponding to $p < 0.005$, uncorrected.

Liu et al., 2015; Tao et al., 2016). The current study found that the tai chi group showed decreased DLPFC-MFG functional connectivity compared to the control group. This result was consistent with recent findings that tai chi practice significantly decreased resting-state functional connectivity between the DLPFC and the frontal regions (Tao et al., 2017). A large body of evidence indicated that the DLPFC and the MFG were key regions of the executive control network playing important roles in top-down cognitive control processes such as intellectual performance (Hopfinger et al., 2000; MacDonald et al., 2000), impression management (Vohs et al., 2005), and emotion regulation (Compton et al., 2008). Neuroimaging studies on the executive control network and aging suggested that high-performing older adults may compensate for disruption of the cognitive control network by recruiting additional frontal resources to overcome cognitive control deficits (Gutchess et al., 2007). For example, a study that investigated the effects of a 14-day longevity lifestyle program found that improved brain

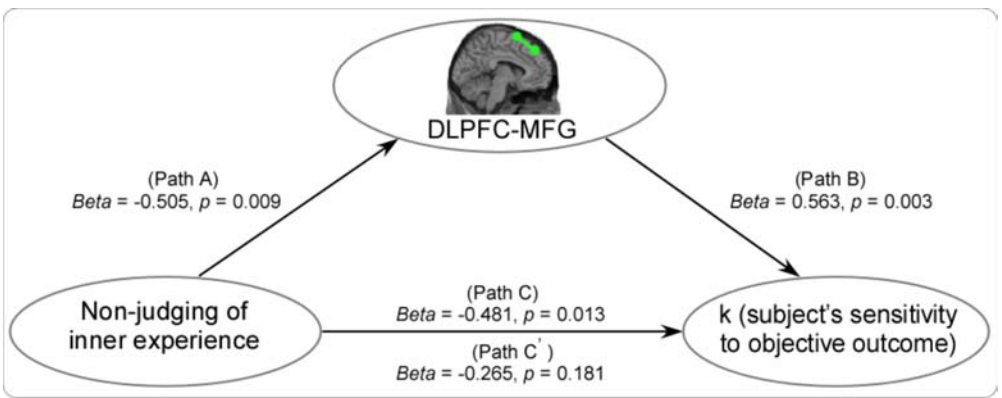

FIGURE 5 | The *K*-value and the functional connectivity between the DLPFC and the right MFG (MNI 38 –6 54) showed a positive correlation in the tai chi group **(A)**. The results showed that the functional connectivity strength between the DLPFC and the MFG was positively correlated with the *K*-value in the tai chi group ($r = 0.563$, $p < 0.01$). No correlation was found in the control group ($r = 0.146$, $p > 0.05$) **(B)**.

TABLE 5 | The correlation between the *K*-value and resting-state functional connectivity in the tai chi group (seed region: DLPFC).

Region		Peak activation			*t*-Value	Voxels
		X	*Y*	*Z*		
Positively correlated with k						
R	Supramarginal gyrus	66	−20	26	6.81	1309
R	Insula lobe	40	−6	0	4.33	
R	MFG	38	−6	54	3.82	
L	Precentral gyrus	−62	−18	30	5.22	401
L	Inferior parietal lobule	−46	−24	38	5.01	
R	Paracentral lobule	10	−32	64	4.85	161
L	Postcentral gyrus	−36	−36	58	5.3	133
Negatively correlated with K						
	No region					

Coordinates (mm) are in MNI space. L, left hemisphere; R, right hemisphere. All of the clusters survived FWE correction ($p < 0.05$) for multiple comparisons at the cluster level, with a voxel-level threshold corresponding to $p < 0.005$, uncorrected.

metabolism was associated with a decrease in the connectivity between the DLPFC and the frontal regions, which was interpreted as a marker of greater cognitive efficiency of this brain region (Small et al., 2006). Moreover, Tang et al. (2015) suggested that novice meditators need to overcome habitual ways of internally reacting to their own emotions and might therefore show an increased recruitment of the prefrontal regions, while experienced meditators (that is, those with long-term tai chi experience) might have automated an accepting stance toward their experience and therefore show weaker prefrontal activation. Hence, the benefits associated with increased activity within the executive control network might be related to compensatory mechanisms rather than indicating a healthy state. In the current study, decreased functional connectivity between the DLPFC and the MFG in older adults with long-term tai chi experience might be a desirable outcome, which suggested the improvement and high efficiency of executive control.

Healthy elders in both the tai chi and control groups were balanced in age, gender, educational years, and personality traits such as depression and impulsivity. Therefore, it was unlikely that the resting-state functional connectivity difference observed in the executive control network between the groups was due to the demographic characteristics. Importantly, the DLPFC-MFG functional connectivity was negatively correlated with non-judgment of inner experience, a key component of meditation, among elders with long-term tai chi experience, indicating that a stronger meditation level was associated with weaker functional connectivity within the executive control network. In fact, many studies have confirmed the positive benefits of meditation such as improving cognitive flexibility (Moore and Malinowski, 2009; Kozasa et al., 2012). For example, Amishi et al. (2007) verified that participants practicing long-term meditation demonstrated greater skills in regulating their executive control attention compared to those in a control group. Teper et al. (2013) found that participants who scored high on the acceptance facet of mindfulness committed fewer errors on a Stroop task, a canonical measure of executive control. The current study extended previous research and suggested that long-term tai chi experience could improve elders' executive control abilities by reducing functional connectivity within the executive control network, which might be related to the non-evaluative stance and acceptance toward inner experiences of tai chi practitioners.

Furthermore, the present study found a relationship between functional connectivity within the executive control network and sensitivity to objective outcomes during the sequential risk-taking task for long-term tai chi practitioners. Specifically, reduced functional connectivity between the DLPFC and the MFG was associated with less sensitivity to objective outcomes, that is, stronger ability of emotion regulation, or strategies that could influence which emotions arise and how these emotions

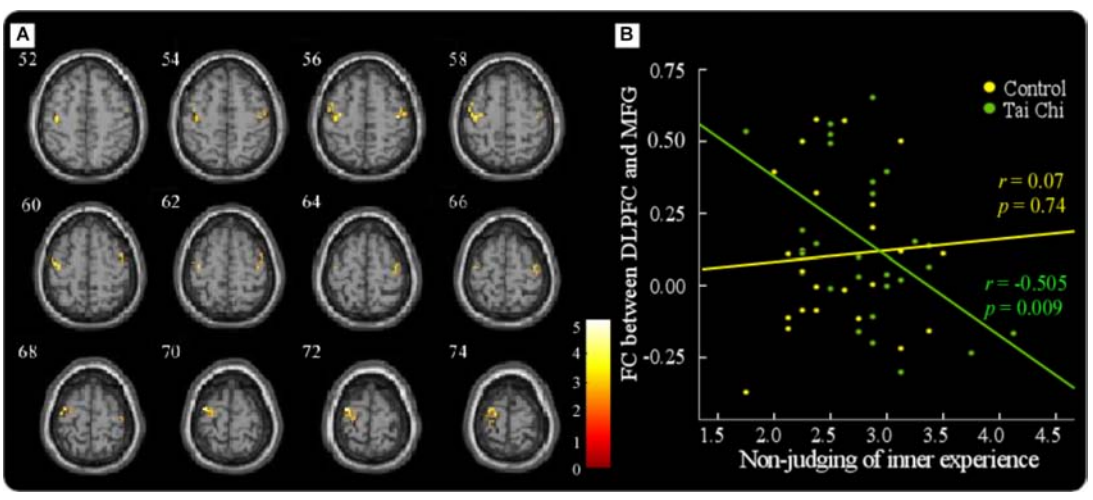

FIGURE 6 | The mediation analysis showed that there was no significant effect of non-judgment of inner experience on the subjects' sensitivity to outcomes (K) after including the functional connectivity between the DLPFC and the MFG (Path A: *beta* = –0.505, $p < 0.01$; Path B: *beta* = 0.563, $p < 0.01$; Path C: *beta* = –0.481, $p < .05$; Path c': *beta* = –0.265, $p > 0.05$).

were experienced and expressed (Gross and Thompson, 2007). Neuroimaging studies indicated that both the DLPFC and the MFG were involved in the top-down control of emotion regulation (MacDonald et al., 2000; Liu Z. et al., 2017). Notably, the current study also revealed that the functional connectivity between the DLPFC and the MFG in the tai chi group fully mediated the impact of non-judgment of inner experience on their sensitivity to outcomes. Previous studies failed to measure the relationship among meditation, functional connectivity within the executive control network, and emotion-regulation ability. To the best of our knowledge, this was the first imaging study showing that for long-term tai chi practitioners, the modulation of non-judgment of inner experience on their emotion regulation was achieved through decreased functional connectivity between the DLPFC and the MFG.

LIMITATIONS

This study did not mention the exercise intensity of the control or tai chi groups. As tai chi combines meditation with physical exercise of light-to-moderate intensity, it is possible that the exercise intensity in the control group was much higher than in the tai chi group even if the exercise time per day was similar between these two groups. The difference in exercise intensity between the tai chi and control groups may have contaminated the research results. This study's argument will be strengthened by clarifying if the intensity and modality of the exercises practiced by the controls were similar to the physical components of tai chi.

CONCLUSION

The current resting-state fMRI study aimed to examine whether the impact of the meditative component of tai chi on emotion regulation was mediated by functional connectivity within the executive control network. Behaviorally, for older adults, long-term tai chi experience can improve both non-judgment of inner experience and emotion-regulation ability. Moreover, the non-judgment of inner experience of the tai chi practitioners was positively correlated with their emotion-regulation ability. On the neural level, long-term tai chi experience could reduce the functional connectivity between the DLPFC and the MFG, which was a reflection of stronger executive control function in elders. Interestingly, in older adults with long-term tai chi experience, the functional connectivity between the DLPFC and the MFG fully mediated the impact of non-judgment of inner experience on their emotion-regulation ability. The current study showed that the modulation of non-judgment of inner experience on long-term tai chi practitioners' emotion regulation was achieved through decreased functional connectivity within the executive control network.

AUTHOR CONTRIBUTIONS

XG, ZL, and LL designed the experiments, assisted with the interpretation of the data, and wrote the manuscript. ZL and YW programmed the experimental scenario, conducted the experiments, and analyzed the data. All of the authors read and approved the final version of the manuscript for submission.

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Ratings of Perceived Exertion Misclassify Intensities for Sedentary Older Adults During Graded Cycling Test: Effect of Supramaximal High-Intensity Interval Training

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The present study aims (1) to evaluate ratings of perceived exertion (RPE) and corresponding intensities during a maximal graded cycling test and (2) to determine the effects of 6 weeks of supramaximal cycling exercise (SCE) intervention on RPE and associated physiological factors in young and older sedentary groups. Two healthy groups of 17 young adults [average (SD) age: 26.2 (2.4) year] and 13 older adults [average (SD) age: 54.5 (2.3) year] completed a 6-week SCE intervention on an ergocycle. Physiological values and RPE were collected across stages corresponding to ventilator thresholds 1 (VT1) and 2 (VT2) of the graded cycling test and 10 min following the end of test and during the six bouts of SCE. The relative intensity for both VT1 and VT2 were also objectively calculated based on the percent of maximal heart rate %HRmax and peak oxygen consumption %$\dot{V}O_2$peak.

Before SCE intervention, RPE values were significantly higher for the older group compared to younger at VT1 [$p < 0.01$] and VT2 [$p < 0.01$], although both groups were working at similar relative intensities (%$\dot{V}O_2$). After 6 weeks of SCE, the older group's perceived effort values were normalized to the actual estimated ones and were similar to those observed in younger individuals. The intervention elicited physiological changes at rest and submaximal intensities, while no improvements were noted for both groups in aerobic fitness (i.e., $\dot{V}O_2$peak). For both groups, RPE decreases with SCE at 10 min following graded test correlated significantly to $\dot{V}O_2$ ($r = 0.61$, $p < 0.01$). Our study revealed that the initial over-estimation of the exertion levels found for the older sedentary group at the tested submaximal intensities was no longer present after 6 weeks of SCE training, therefore matching RPE values of the young group and those estimated by %HRmax and %$\dot{V}O_2$peak methods. Therefore, combining the RPE method with other commonly used methods of estimating exercise intensity is highly recommended for sedentary older adults to suitably monitor the exercise intensity.

Keywords: older individuals, rating of perceived exertion, ventilatory threshold, sedentary, supramaximal cycling exercise

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INTRODUCTION

As the world's population is aging with most countries experiencing an increase in the portion of older adults, there is a growing need to prevent morbidity and improve the quality of life with advancing age. Exercise is an effective and valuable alternative to provide health benefits and prevent from multiple deleterious effects of aging (Hale and Marshall, 2017; Reid and Foster, 2017). Many recommendations are available today to guide exercise prescription for health and disease. The most commonly used guideline refers to 30 min of moderate-intensity aerobic (endurance) physical activity on 5 days each week or 20 min of vigorous-intensity aerobic physical activity on 3 days each week to promote or maintain health (Haskell et al., 2007). However, several barriers to exercise have revealed the importance of adherence and an appropriate administration of the prescribed exercise (e.g., intensity).

A little more than a decade ago, exercise scientists have started exploring the benefits of high-intensity interval training (HIIT) characterized by brief, intermittent bouts of vigorous-intensity exercise, interspersed by periods of rest or active recovery (Gibala et al., 2012). This relatively new method of training is showing comparable outcomes to those of a traditional moderate-intensity longer-duration exercise (Gibala et al., 2009). HIIT has proven to be an efficient alternative to promote aerobic fitness as well as several health parameters over brief periods of time (Gillen and Gibala, 2013) or even with a single session per week (Matsuo et al., 2014). Despite the potential benefits of HIIT, some studies reported that such high-intensity model of training induced discomfort, which is deterrent to long-term adherence (Ekkekakis et al., 2011; Foster et al., 2015). At the contrary, other studies showed that HIIT represents an attractive training strategy compared with continuous aerobic exercise (Jung et al., 2015) and might be achieved with a lower rate of perceived effort (Bangsbo et al., 2015). Perhaps the most salient characteristic of HIIT remains its time-efficiency. Chao et al. (2000) have reported that older adults perceive exercise participation to be overly time-consuming, which represents a critical barrier to any exercise program adherence.

It has been recently acknowledged that most studies on HIIT were performed on young adults and that more focus should be given to older participants in order to better understand the effects of HIIT on that portion of the population (Weston et al., 2014). HIIT remains a multimodal training with a broad potential of application. Each HIIT form can differ significantly from others depending on the duration and intensity of each interval, the number of intervals performed, and the duration of recovery (relative to the effort time) between bouts of effort. Different HIIT protocols have shown varying physiological and psychological adaptive responses (Ross and Leveritt, 2001). To date, the most common exercise that has been used in obesity management for instance, is the Wingate test. The latter consisting of 30 s of all-out sprinting induced many beneficial effects in overweight individuals (Whyte et al., 2010), however, this protocol remains extremely difficult, and participants have to tolerate some substantial discomfort. More recently, Jabbour et al. (2016) reported that very brief (i.e., 6 s) high-intensity exercise in the form of sprinting induced substantial improvements in both performance and health-related outcomes in similar obese participants. In addition, this form of supramaximal and very brief exercise is motivating and tolerated well by participants, which is reflected by the excellent compliance to the intervention. These results highlight the potential of this exercise model to provide an alternative exercise intervention for the improvement of health among populations with or at risk of health problems. Moreover, monitoring adequately exercise load to determine whether an individual is appropriately targeting the prescribed exercise training can promote effectiveness of the intervention especially among a very susceptible population such as sedentary older individuals.

Today, there is a number of potential indicators used to increase our understanding of the exercise load and its effect on the individual. The rating of perceived exertion (RPE) is one of the most popular and reliable tools that provides an understanding of physiological stress during exercise as well as retrospective information regarding perceived effort during exercise (Halson, 2014). Perceived exertion is a psycho-physiological marker of intensity resulting from a complex integration of subjective feelings of effort, strain, discomfort, and/or fatigue experienced during exercise (Robertson and Noble, 1997). To optimize exercise-derived health benefits in older individuals it seems necessary that the prescribed exercise be tailored and individualized according to reported subjective measures of intensity. For healthy sedentary and special populations (e.g., persons with cardiovascular or metabolic diseases) HIIT has been used (Whyte et al., 2010; Gillen and Gibala, 2013; Jabbour et al., 2016) and often prescribed based on heart rate and oxygen uptake responses during incremental exercise testing (Coquart et al., 2008; Akbarpour, 2013; Biddle and Batterham, 2015). While these methods require sophisticated equipment and laboratory spaces, the use of RPE presents an important advantage for prescribing and self-regulating the effort in a real-exercise setting. In healthy middle-aged and elderly individuals, RPE was shown not to be impaired by aging and to be associated with heart rate to control exercise intensity (Sidney and Shephard, 1977; Aminoff et al., 1996). Interestingly, Grange et al. (2004) did not find a significant relationship between RPE and HR in physically deconditioned older persons (75.2 years). However, following 6 weeks of arm training, a significant HR-RPE relationship was found in most of the subjects. For non-elderly individuals other variables were also combined with RPE (e.g., oxygen uptake, ventilator threshold) to assess and regulate the intensity of exercise. While much is known about RPE responses and related factors in children and adults, little is known about the perceptual responses to exercise (i.e., RPE) or associated factors in older sedentary members of the population. Whether RPE can be used appropriately and accurately in such groups needs to be further explored given their different response and tolerance to exercise, in order to be more widely used in the health and clinical sectors.

With the growing interest of promoting exercise prescription among elderly population, optimizing the health benefits as well the adherence to exercise programs remains the major concern. Despite the promising HIIT and sprint interval training (SIT)

results, no study has yet examined the changes in perceptual responses (i.e., RPE) following supramaximal HIIT (SIT) and their relationship with physiological outcomes in a sedentary population of young and older adults. Assuming that the RPE assesses adequately the exercise intensity for older and younger individuals, the present study aims to compare RPE and associated factors in young and older sedentary individuals before and after 6 weeks of supramaximal HIIT on ergocycle, more commonly known as supramaximal cycling exercise (SCE). For the purpose of the current work, RPE and physiological responses were evaluated at different relative submaximal and maximal intensities and after 10 min of recovery during two maximal graded cycling tests performed before and after the SCE program. RPE and physiological responses were also examined immediately at the completion of two six bouts of supramaximal cycling tests realized at the start and end of the SCE program. We hypothesized that RPE presents an interesting tool to estimate suitably the exercise intensity for older individuals and SCE intervention improves RPE concomitantly with the improvement of physiological and fitness variables as reported for adults.

MATERIALS AND METHODS

Participants

Seventeen healthy young adults [12 female and 5 male; average (SD) age = 26.2 (2.4) years old] and thirteen healthy old adults [8 female and 5 male; mean (SD) age = 54.5 (2.3) years old] volunteered for the study. Participants were classified according to growth stages: young (18–40 years) and older adults (41–71 years). The sample consisted of university students and staff who were recruited through posted announcements. Physical characteristics of both groups are presented in **Table 1**. The inclusion criteria for participation were as follows: (i) be sedentary [$<$60 min.week^{-1} of structured exercise, as assessed by the International Physical Activity Questionnaire (Craig et al., 2003)], (ii) not be taking part in any systematic exercise training at the time of study or during the 6 months that preceded the study, (iii) no history of orthopedic, neurological, cardiovascular or other chronic disease, (iv) no history of drug consumption or (v) smoking. The procedures were approved by the University's Human Research Ethics Committee (UHRC), and performed in accordance with the Helsinki Declaration of 1975, as revised in 2008. Informed consent was obtained from all participants prior to start of the study.

Protocol

The protocol consisted of two testing sessions performed before (pre-intervention) and after (post-intervention) a 6-week supramaximal HIIT program on a cycle ergometer, referred to here as supramaximal cycle exercise (SCE). Pre-intervention testing sessions determined baseline levels of key variables, while the post-intervention testing sessions examined changes in key values inferred by the SCE program. Each of the pre- and post-intervention testing sessions followed the same procedures and were conducted on two different days (Day 1 and Day 2) separated by a minimum of 48 h, and took place in the morning of each day ($\sim$ 8.30am) after an overnight fast. For the testing sessions, non-menopausal female participants were in the follicular phase of their menstrual cycle. During the 6 weeks of intervention all participants completed all of the training sessions (thus adherence was 100%) and no other difficulties or incidents were encountered. Two supramaximal cycling tests were further conducted at the first and last SCE sessions as part of the training. The current protocol has been developed and previously used by our team (Jabbour et al., 2018). Before starting the experiment, participants were thoroughly familiarized with the equipment and testing procedures and were instructed on how to indicate the RPE (6–20 Borg scale, Borg, 1970) when requested by the experimenter. The range of sensations that correspond to categories of effort within the scale were clearly explained to each participant.

Pre- and Post-intervention Testing Sessions

At baseline on Day 1 and after assessing body composition, participants performed an incremental maximal test on a cycle ergometer (Ergomedic 839E, Monark, Sweden) with continuous measurement of pulmonary gas exchange using a breath-by-breath automated metabolic system (Ergocard MEDI-SOFT, Sorinnes, Belgium) to determine peak oxygen consumption ($\dot{V}O_2$peak). Calibrations were performed prior to each test using standard gasses of known oxygen and carbon dioxide concentrations as well as a calibration syringe for air flow. Before beginning the test, participants remained seated for 5 min on the bicycle ergometer in the same position as that used for exercise to measure resting values. The test began at an initial power of 25 watts and increments of 25 watts followed every 5 min until exhaustion. During the test, participants were instructed to pedal at a rate of 50–70 revolutions per minute. The test was terminated when the participants requested to stop the exercise or could no longer maintain the required pedaling rate ($<$40 revolutions per minute). A recovery phase of 5 min at 25 watts followed the test (Jabbour et al., 2018).

After a 48-h rest on Day 2 and following a 10-min warm-up, participants performed a Force-Velocity test on a cycle ergometer using a technique adapted from the study of Vandewalle et al. (1988). This test consists of a succession of supramaximal bouts of approximately 6 s, with flywheel resistance increasing by 1 kg after each bout until the subject is unable to perform the test. A period of passive recovery (5 min) was allowed between successive bouts. The peak velocity for each bout was recorded, and the power output was calculated by multiplying the load with the speed. The optimal load corresponded to the load at which maximal power (Pmax) was achieved. This load was then used for the SCE protocol that followed. The Force-Velocity test was also performed every 2 weeks to adjust the individual power level used during SCE.

SCE Intervention and Supramaximal Tests

Once participants completed the preliminary testing, a total of 18 SCE training sessions was prescribed over a period of

TABLE 1 | Results on anthropometric data before (pre-intervention) and after (post-intervention) the supramaximal cycling exercise (SCE) intervention for both young and older groups.

	Pre-intervention		Post-intervention		Interaction Effect	
	Young	Older	Young	Older	*F*	*p*
Height *(m)*	1.69 (1.1)	1.68 (1.3)	1.71 (1.1)	1.68 (1.3)	1.6	0.33
Body mass *(kg)*	82.3 (3.8)	90.1 (2.8)[a]	82.2 (1.1)	87.1 (3.3)[ab]	11.6	<0.01
BMI *($kg.m^{-2}$)*	28.6 (1.1)	31.9 (1.1)[a]	28.7 (1.4)	30.5 (1.5)[ab]	12.7	<0.01
FM *(kg)*	27.1 (1.1)	33.8 (2.6)	26.4 (1.7)	31.8 (2.9)[ab]	20.1	<0.01
FFM *(kg)*	56.1 (2.1)	56.2 (1.1)	57.1 (2.1)	56.1 (1.3)	0.4	0.63

Values are mean ± SE (standard error). BM, body mass; BMI, body mass index; FM, fat mass; FFM, fat free mass. [a]Significant difference between groups ($p < 0.01$). [b]Significant difference from pre-intervention values within an age group ($p < 0.01$).

6 weeks (three sessions per week). The same training protocol has been previously developed and tested by our laboratory (Jabbour and Iancu, 2015; Jabbour et al., 2015, 2018). Each of the prescribed sessions began with a 5-min warm-up consisting of continuous cycling at moderate intensity corresponding to 40–50% of each participant's maximal heart rate (HRmax), and was followed by 6 repetitions of SCE intervals with 2 min of passive recovery between each repetition. Each SCE repetition lasted 6 s, and participants were asked to pedal at maximal velocity against the resistance that was determined on Day 2. Heart rate values were monitored during all training sessions using a heart rate monitor (Polar, Kempele, Finland). The total duration of each session was approximately 16–18 min. During the training sessions, the velocity (in revolutions per minute) was recorded for each second of the entire round to ensure that participants pedaled at their maximal capacity. Indeed, values varied among individual in range of ∼160–200 rpm.

Additional testing was also completed during the first and the last (18th) SCE training sessions. Indeed, during these two testing sessions, participants were asked to perform one of their regular training sessions, while power output and heart rate values obtained for the 6 SCE repetitions were recorded.

Data Analysis

In the present work, the training adherence of the participants was calculated as the percentage of the actual number of training sessions completed in compliance with the targeted intensity and duration, relative to the total number of training sessions prescribed.

Anthropometric Data

Body mass, fat-free mass and fat mass were assessed using bio-impedance scale (Bodystat1500, Isle of Man, British Isles). Height was determined to the nearest 0.5 cm with a measuring tape affixed to the wall. Body mass index (BMI) was calculated as the ratio of mass (kg) to height squared (m^2).

Pre- and Post-intervention Testing Data

For $\dot{V}O_2$peak tests, ventilatory and gas exchange data were collected on a breath-to-breath basis along with continuous heart rate (HR, $beats.min^{-1}$) measurements. Data on minute ventilation $\dot{V}_E$, ($L.min^{-1}$), oxygen consumption ($\dot{V}O_2$, $mL.min^{-1}$), carbon dioxide production ($\dot{V}CO_2$, $mL.min^{-1}$) and respiratory exchange ratio (RER) were determined at each increment level as the average of the last 20 s where a steady-state in values was reached. Systolic (SBP) and diastolic (DBP) blood pressure (mmHg) were measured at the left arm using the auscultatory method with a stethoscope and sphygmomanometers (Vaquez-Laubry, Spengler, Issoudun, France) and respectively averaged over three recordings. Ratings of perceived exertion (RPE, Borg, 1970) were collected at the end of each increment level by asking participants to raise their arm to indicate the RPE value as the experimenter read up the Borg scale. The latter ranged from 6 to 20 with 7 indicating that the effort is *very very light*, and 19 indicating that the effort is *very very hard*.

For the purpose of the analysis, $\dot{V}O_2$, HR, SBP, DBP, and RPE were reported at 5 different moments of the pre- and post-intervention's maximal incremental cycling tests: (i) prior to start at rest (except for RPE), (ii) at the first and (iii) second ventilatory thresholds (VT1 and VT2), (iv) at the maximal workload ($\dot{V}O_2$peak) and (v) 10 min after the completion of the test (recovery). Peak oxygen consumption ($\dot{V}O_2$peak) was determined using the following criteria: (1) a peak or plateau in oxygen uptake values despite an increase in exercise intensity, (2) respiratory exchange ratio $\geq$ 1.1, (3) peak heart rate $\pm$ 10 $beats.min^{-1}$ of the predicted maximal heart rate (220 - age) and (4) voluntary exhaustion indicated by an RPE > 17 (Spiro, 1977; Howley et al., 1995). In the present study, $\dot{V}O_2$peak was determined as $\dot{V}O_2$ mean of the final 20 s of each stage, and the $\dot{V}O_2$peak was assumed as the highest $\dot{V}O_2$mean reached in incremental maximal test (Malta et al., 2018).

Ventilatory thresholds were determined using established criteria as per Wasserman et al. (1999) and used to classify the intensity of aerobic exercise. Briefly, VT1 corresponds to the break point in the plot of $\dot{V}CO_2$ as a function of $\dot{V}O_2$. At that point, $\dot{V}_E/\dot{V}O_2$ increases without an increase in $\dot{V}_E/\dot{V}CO_2$. VT2 was located between VT1 and $\dot{V}O_2$peak when $\dot{V}_E/\dot{V}O_2$ begins to increase and $\dot{V}_E/\dot{V}CO_2$ continues to increase. VT1 and VT2 were determined independently by three experienced investigators. At these two stages, we determined the relative intensity corresponding to the percentage of maximal heart rate (%HRmax) and to the percentage of peak oxygen consumption (%$\dot{V}O_2$peak) (Garber et al., 2011).

As for the supramaximal testing, power output (P, W.kg^{-1}), HR, %HRmax and RPE were collected for each of the six repetitions of the first and last SCE training sessions. RPE was obtained immediately after the end of each 6-s interval.

Statistical Analyses

Before the analysis, all datasets were tested for normality using the Kolmogorov–Smirnov test. ANOVAs with 2 × 2 repeated measures [Intervention (pre- and post-HIIT intervention) × Group (young and older)] were performed on all variables (anthropometric, physiological, RPE and power output) collected for the 5 key moments of the incremental maximal test as well as during each of the 6 repetitions of the supramaximal testing. When a significant interaction effect was found, the analysis was completed with Bonferroni's *post hoc* for pairwise comparisons. Pearson correlations were used to assess the association between RPE and anthropometric, physiological and fitness variables. The analyses were performed using IBM SPSS Statistics 19 software (IBM SPSS Statistics for Windows, Version 24.0. Armonk, NY, United States: IBM Corp.). A value of $p < 0.05$ was considered statistically significant for all tests.

RESULTS

Of the 30 eligible participants, none withdrew from the intervention. Accordingly, the data provided from 100% of the total sample was used for subsequent analyses.

Anthropometric Variables

ANOVA's results on anthropometric variables are presented in **Table 1**. At the pre-intervention test, an initial significant difference in body mass and BMI values was detected between the young and older groups and maintained at post-intervention test. The (intervention × group) interaction effect reported on body mass, BMI and FM was explained by the significant decrease in values following the intervention only for the older group, while no significant changes were incurred by the SCE program for the young group.

Incremental Maximal Tests

During the maximal incremental cycling test, VT1 and VT2 were attained by both groups at similar relative intensities that did not vary significantly with the 6-week SCE intervention (**Table 2**).

Physiological Variables

ANOVAs' results on physiological variables are presented in **Table 3**. In brief, at the pre-intervention phase and as compared to the young group, the older group had significantly higher values for $\dot{V}O_2$, SBP and DBP mainly at rest and during VT2, while no other differences were noted at VT1, maximal workload (to the exception of SBP) or during recovery as compared to the young group. The SCE intervention improved significantly the $\dot{V}O_2$ and SBP values for both groups at rest, VT1, VT2 and during recovery, while no intervention-related changes were found for physiological variables at the maximal workload. Furthermore, the older group improved their DBP after the SCE training to reach similar resting values as those of the younger group. The intervention also resulted in both groups improving their recovery values for HR. Moreover, the initial between-group differences in $\dot{V}O_2$ and SBP at VT2 were no longer significant at post-intervention, while significant differences in groups were still seen for $\dot{V}O_2$ at rest and for SBP at rest and at the maximal workload following the intervention.

Rating of Perceived Exertion

Table 3 presents ANOVAs' results on RPE. At the pre-intervention phase, the older group perceived a significantly higher effort at the relative submaximal intensities VT1 and VT2 as compared to the young group. Indeed, at VT1 the older group judged the effort as being *very hard* compared to a *fairly light* rating for the young group, knowing that the actual relative intensity did not differ between groups (49–51%$\dot{V}O_2$peak). This was also the case at VT2, where the older group judged the effort as being *very very hard* compared to a *somewhat hard to hard* for the young group, while the actual relative intensity was similar between groups (80%$\dot{V}O_2$peak). The intervention resulted in a significant decrease in the RPE values for both groups at the recovery phase (10-min post-exercise) from *very hard* to *somewhat hard to hard*. RPE only decreased significantly for the older group at both relative submaximal intensities thus bringing their perception of effort to the same levels as those seen in the young group (VT1: *fairly light*; VT2: *somewhat hard to hard*). At $\dot{V}O_2$peak, RPE values reached their maximal level for both groups.

At 10 min post-graded cycling test (recovery), RPE decreases correlated positively with $\dot{V}O_2$ for both groups ($r = 0.61$; $p < 0.01$). However, no significant associations were found between RPE and both post-intervention BM and HR decreases.

Supramaximal Tests

After the SCE intervention, no significant changes were obtained for RPE values among groups compared with those observed at baseline. The RPE values corresponded to *fairly light* for both groups. During SCE, the relative intensity was 54% of maximal heart rate for both groups at baseline and at post-intervention (**Table 4**). In this study, the RPE obtained for both groups during the six bouts of SCT at baseline and at post-intervention correlated significantly with HR values ($r = 0.61$; $p < 0.01$). No significant correlation was obtained between RPE and muscle power increases observed for both groups at post-intervention ($r = 0.002$; $p = 1.1$).

DISCUSSION

The first aim of the present study was to examine differences in the subjective sense of effort in relation to objective measures of intensities and associated physiological responses during maximal graded and SCE in sedentary young and

older individuals. The second objective was to determine potential benefits of 6-weeks of supramaximal HIIT (i.e., SCE) intervention in such groups on the sense of effort as an intensity regulator. At the best of our knowledge, this study is the first to evaluate RPE during graded cycling test and during bouts of SCE as well as the effect of 6 weeks of SCE on RPE, and associated factors among young and older sedentary adults. The primary finding of the present work is the inconsistency found at baseline in perceived exertion between young and older participants

TABLE 2 | Results on relative intensities reached at the first and second ventilatory thresholds (VT1 and VT2) before (pre-intervention) and after (post-intervention) the supramaximal cycling exercise (SCE) intervention for both young and older groups.

		Pre-intervention		Post-intervention		Interaction effect	
		Young	**Older**	**Young**	**Older**	***F***	***p***
VT1	%HRmax	54 (3)	58 (4)	54 (4)	54 (1)	3.1	0.28
	%$\dot{V}O_2$peak	51 (4)	49 (9)	48 (3)	49 (5)	2.8	0.27
VT2	%HRmax	80 (2)	80 (1)	77 (4)	75 (8)	3.2	0.29
	%$\dot{V}O_2$peak	69 (3)	73 (4)	62 (3)	64 (7)	2.4	0.25

Values are mean ± SE (standard error). VT1 and VT2, ventilatory thresholds 1 and 2; %HRmax, percentage of maximal heart rate; %$\dot{V}O_2$peak, percentage of peak oxygen consumption. [a]Significant difference between groups ($p < 0.05$). [b]Significant difference from pre-intervention values within an age group ($p < 0.05$).

TABLE 3 | ANOVA results for the intervention × group interaction effects on physiological and perceptual data analyzed at rest, during the first and second ventilator thresholds (VT1 and VT2), at maximal workload and 10 min after the end of the test (recovery).

	Pre-intervention		Post-intervention		Interaction effects	
	Young	**Older**	**Young**	**Older**	***F***	***p***
Rest						
$\dot{V}$ O_2 *(mL.min^{-1})*	616.2 (21)	730.4 (15)[a]	580.2 (18)[b]	610.6 (16)[ab]	11.8	< 0.01
HR (beats.min^{-1})	75 (8)	76 (8)	76 (5)	78 (6)	1.18	0.56
SBP*(mmHg)*	113 (9)	116 (12)	107 (6)[b]	110 (11)[ab]	7.9	< 0.01
DBP*(mmHg)*	74 (8)	82 (10)[a]	72 (7)	75 (3) [b]	14.3	< 0.01
VT1						
$\dot{V}$ O_2 *(mL.min^{-1})*	1149.4 (55) (63)	1168.7 (70)	1045.6 (63)[b]	1140.6 (60)[b]	21.2	< 0.01
HR *(beats.min^{-1})*	101 (4)	104 (3)	100 (3)	102 (3)	1.1	0.23
SBP*(mmHg)*	171 (9)	175 (4)	150 (10)[b]	155 (7)[b]	9.8	< 0.01
DBP*(mmHg)*	78 (6)	84 (6)	76 (6)	79 (3)	3.1	0.53
RPE	11 (0.3)	17 (0.3)[a]	11 (0.1)	11 (0.3)[ab]	12.8	< 0.01
VT2						
$\dot{V}$ O_2 *(mL.min^{-1})*	1540.3 (91)	1717.5 (114)[a]	1434.3 (95)[b]	1490.7 (136)[b]	11.2	< 0.01
HR *(beats.min^{-1})*	151 (4)	135 (6)	145 (4)	124 (5)	1.1	0.23
SBP*(mmHg)*	175 (16)	185 (18)[a]	169 (13)[b]	173 (4)[b]	9.2	< 0.01
DBP*(mmHg)*	82 (7)	84 (7)	81 (8)	83 (8)	2.9	0.66
RPE	14 (0.3)	19 (0.6)[a]	14 (0.2)	14 (0.3)[b]	11.2	< 0.01
Maximal Workload						
$\dot{V}$ O_2peak *(mL.min^{-1})*	2221.7 (485)	2338 (511)	2278(580)	2298 (380)	3.1	0.63
HRmax *(beats.min^{-1})*	187 (3)	177 (4)	188 (4)	179 (5)	5.1	0.33
SBP *(mmHg)*	189 (14)	170 (11)[a]	186 (9)	169 (15)[a]	10.01	< 0.01
DBP *(mmHg)*	87 (3)	89 (4)	86 (8)	88 (6)	1.8	0.11
RPE	20 (0.3)	20 (0.3)	20 (0.2)	20 (0.3)	1.8	0.11
Recovery						
$\dot{V}$ O_2 *(mL.min^{-1})*	1749.6 (63)	1768.8 (70)	1445.4 (63)[b]	1440.5 (60)[b]	11.01	< 0.01
HR*(beats.min^{-1})*	150 (4)	155 (3)	134 (3)[b]	135 (3)[b]	21.01	< 0.01
SBP*(mmHg)*	161 (9)	162 (4)	151 (10)[b]	153 (7)[b]	11.2	< 0.01
DBP*(mmHg)*	79 (6)	81 (6)	78 (8)	81 (3)	3.8	0.63
RPE	17 (0.3)	17 (0.3)	12 (0.2)[b]	12 (0.3)[b]	19.8	< 0.01

Values are mean ± SE (standard error). HR, heart rate; $\dot{V}$ O_2, oxygen consumption, $\dot{V}$ O_2peak, peak oxygen consumption, SBP: systolic blood pressure, DBP: diastolic blood pressure, RPE: rating of perceived exertion. [a]Significant difference between groups ($p < 0.01$). [b]Significant difference from pre-intervention values within an age group ($p < 0.01$).

at relative submaximal exercise intensities. Only the older group perceived the effort as being significantly higher as compared to the actual exercise intensity at VT1 and VT2 determined by objective methods. The 6-week SCE training program helped in normalizing perceived exertion values in the older group, bringing their RPE values determined at VT1 and VT2 to an adequate match of the actual exercise intensity classified by both $\%\dot{V}O_2$peak and %HRmax methods.

At baseline, the RPE values were significantly higher in older group compared to adults at the same relative exercise intensities corresponding to VT1 and to VT2 of the graded cycling test. In fact, in accordance to the Borg scale for ratings of perceived exertion (Borg, 1970) the older individuals perceived the two exercises stage (VT1 and VT2) as being *very hard* and *very very hard*. According to American College of Sport Medicine for intensity classification (Garber et al., 2011), the relative intensity determined in the present work using %HRmax and $\%\dot{V}O_2$peak methods correspond to *fairly light* for VT1 and to *somewhat hard* for VT2 which does not correspond adequately to the intensity perceived by RPE for the older group. At the contrary, young adult participants perceived VT1 and VT2 as *fairly light* and *hard* respectively corresponding with ratings observed by Alberton et al. (2016) that indicate values near to 16–17 when targeting a VT2 intensity for young women.

The discrepancy observed for RPE between our two groups may be explained by the age that has the potential to influence RPE as previously reported. Actually, some authors have reported that with aging many factors impair the cognitive functions among elders leading to alter their perceived exertion (Chodzko-Zazko and Moore, 1994; Boutcher, 2000). Accordingly, Grange

TABLE 4 | Results on measured variables during the 6 repeated supramaximal cycling exercises at the first (pre-intervention) and the last (post-intervention) HIIT session for both young and older groups.

	Pre-intervention		Post-intervention		Interaction effect	
	Young	Older	Young	Older	*F*	*p*
1st repetition						
P1 *(W.kg^{-1})*	6.2 (0.3)	6.2 (0.5)	8.5 (1)[b]	7.5 (0.5) [ab]	14.01	< 0.01
HR *(beats.min^{-1})*	104 (3)	102 (2)	104 (3)	102 (2)	4.1	0.23
%HRmax	54	55	54	54	2.1	0.23
RPE	11 (0.2)	11 (0.6)	11 (0.2)	11 (0.6)	1.7	0.54
2nd repetition						
P *(W.kg^{-1})*	6.4 (0.1)	6.3 (0.1)	8.3 (1)[b]	7.1 (0.3)[ab]	14.18	< 0.01
HR *(beats.min^{-1})*	103 (3)	101 (1)	105 (3)	103 (2)	1.2	0.13
%HRmax	54	55	54	54	1.7	0.23
RPE	11 (0.1)	11 (0.6)	11 (0.2)	11 (0.3)	1.9	0.35
3rd repetition						
P *(W.kg^{-1})*	6.1 (0.9)	6.3 (0.6)	8.2 (0.9)[b]	7.8 (0.2)[ab]	13.1	< 0.01
HR *(beats.min^{-1})*	103 (3)	101 (1)	105 (1)	104 (1)	3.1	0.33
%HRmax	53.5	54	54	55	2.1	0.21
RPE	11 (0.2)	12 (0.7)	12 (1.2)	11 (0.6)	1.1	0.54
4th repetition						
P *(W.kg^{-1})*	6.4 (0.3)	6.5 (0.5)	8.5 (1)[b]	7.7 (0.2)[ab]	11.7	< 0.01
HR *(beats.min^{-1})*	106 (3)	104 (2)	104 (3)	104 (1)	1.8	0.16
%HRmax	56	56	54	55	1.7	0.23
RPE	11 (0.4)	11 (0.6)	11 (0.2)	12 (0.2)	1.2	0.15
5th repetition						
P *(W.kg^{-1})*	6.5 (0.2)	6.4 (0.2)	8.6 (1)[b]	7.7 (0.5)[ab]	15.4	< 0.01
HR *(beats.min^{-1})*	105 (3)	103 (2)	103 (3)	103 (2)	1.9	0.18
%HRmax	54	55	54	54	1.6	0.22
RPE	11 (0.4)	11 (0.6)	11 (0.4)	11 (0.6)	1.1	0.12
6th repetition						
P *(W.kg^{-1})*	6.6 (0.1)	6.2 (0.5)	8.5 (1)[b]	7.5 (0.5)[ab]	21.41	< 0.01
HR *(beats.min^{-1})*	104 (3)	102 (2)	104 (3)	102 (2)	1.9	0.18
%HRmax	54	55	54	54	1.6	0.22
RPE	11 (0.2)	11 (0.6)	11 (0.3)	11 (0.3)	1.1	0.12

Values are mean ± SE (standard error). P, power developed at each repetition; 1, 2, 3, 4, 5, 6, number of repetition; HR, heart rate; %HRmax, percentage of maximal heart rate; RPE, rating of perceived exertion. [a]Significant difference between groups ($p < 0.01$). [b]Significant difference from pre-intervention values within an age group ($p < 0.01$).

et al. (2004) did not find any association between RPE and other physiological indicators (e.g., HR) during the course of a graded arm test to maximal exertion among inexperienced older group. At the contrary, Sidney and Shephard (1977) and Aminoff et al. (1996) reported that RPE is not impaired by aging and can be used as tool to control exercise intensity in healthy middle-aged and elderly individuals. For these authors perceived exertion is more affected by the physical fitness and the health status of the subject than by aging alone. In the present study, both our groups were sedentary and did not present previous exercise experiences, which could be revealing of an interaction effect of age and sedentary behavior on perceived exertion, given that only the older group presented altered sensory cues in the perception of effort. In such cases, to provide an accurate assessment and alternatively an appropriate individual exercise prescription, there is a need to combine RPE method with other commonly used methods of estimating relative intensity (e.g., HR) for older sedentary adults. Furthermore, introducing an acclimation period prior to any intervention should be considered among such a group to avoid any intensity misclassifications.

After 6 weeks of SCE, the RPE decreased significantly compared to baseline for the older group at VT1 and VT2 and was similar to those obtained for the young participants. Indeed, the two groups perceived the intensity as being *somewhat hard to hard*. This rate correspond adequately to what has been previously reported on the basis of %HRmax and %$\dot{V}O_2$peak methods (Garber et al., 2011). The normalization of these values observed for older group at VT1 and VT2 seems to be mainly linked to significant improvements of older group's ability to perceive effort since no significant associations were found with physiological indicators and no improvements in aerobic fitness were noted. These results lead us to suggest that training might have increased the subject's abilities to perceive effort, which could be due to an improvement in memory or in the neuromuscular factors (Faulkner et al., 2008) given that no significant improvements in RPE values were seen throughout the intervention. Unfortunately, we did not evaluate neurophysiological adaptations nor memory capacities of our individuals. Therefore, considering these variables on further studies will be beneficial in determining the impact of such SCE model on cognitive function (e.g., attention, memory capacity) and on neurophysiological adaptations (e.g., blood flow, neurotransmitters) among older individuals and their relationship with RPE variations.

During the six bout of SCT, the RPE values were similar across groups at baseline and at post-intervention. In fact, both groups perceived the six bouts of SCT as being *fairly light* which might reveal an advantage for using very short bouts (6 s) of intense exercise. Indeed, an important consideration is that none of the participants withdrew from the intervention with a 100% compliance to the protocol throughout the 6 weeks of training. Moreover, participants displayed a constant relative power output during the six bouts of SCT indicative of a lack of actual fatigue at both baseline and during the post-intervention. This result may allow us to consider our model of SCE as a very appealing strategy for sedentary participants acknowledging the many physiological improvements seen at submaximal intensities in the post-intervention as compared to the baseline values in both groups and especially for the older one. Few studies have evaluated RPE during supramaximal HIIT-type intervention and no data is available for older sedentary individuals. However, it appears that both groups did not experience peripheral (i.e., muscular) and/or central (i.e., neural) fatigue during SCE; however, direct evidence to support this assumption is still lacking. In sum, we can conclude that the SCE regime accomplished in this study by repeating six "all-out" 6-s sprints on cycle ergometer favored a positive commitment among participants and seemed to be a desirable approach to adopt among an older sedentary population.

CONCLUSION

To the best of our knowledge, this study is the first to examine RPE changes in response to 6 weeks of short bouts of supramaximal cycling intermittent exercise in young and older sedentary adults. Our analyses revealed that at baseline, the RPE values calculated at VT1 and VT2 for the older group did not correspond adequately to the relative intensity estimated by %HRmax and %$\dot{V}O_2$peak methods. After the SCE intervention, RPE values were normalized and did not differ from the young adults. Careful attention should be paid on individual intensity assessment and monitoring to avoid any issues with negative consequences on exercise adherence. On the other hand, our study reveals that our SCE regime may be an appealing modality to introduce in older sedentary adults as a strategy aimed at improving exercise adherence, many submaximal physiological responses and therefore health status.

AUTHOR CONTRIBUTIONS

GJ contributed to the conception and design of the study and to the data collection. GJ and LM performed data analysis and interpretation, drafted the manuscript, and revised, read and approved the submitted version.

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frontiers in Aging Neuroscience

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Alteration of Metabosensitive Afferent Response With Aging: Exercised versus Non-exercised Rats

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This study was designed to evaluate the effect of aging on the activity of metabosensitive afferent fibers (thin muscle afferents from group III and IV) and to determine if physical activity performed at old age may influence the afferent discharge. Afferents from *tibialis anterior* and *soleus* muscles were recorded on non-exercised rats aged of 3, 6, 12, and 20 months and on animals aged of 12 and 20 months performing a daily incremental treadmill exercise protocol during the last 8 weeks preceding the recordings. Metabosensitive afferent fibers were activated with potassium chloride (KCl) and lactic acid (LA) injections into the blood stream or by muscle electrically-induced fatigue (EIF). Results indicated that aging is associated to a decrease in the magnitude of the responses to chemical injections and EIF. Unfortunately, physical activity did not allow restoring the metabosensitive afferents responses. These results indicate an alteration of the thin afferent fibers with aging and should be taken into account regarding the management of muscle fatigue and potential alterations of exercise pressor reflex (EPR) occurring with aging.

Keywords: age, fatigue, flexor, extensor, electrophysiology, muscle

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INTRODUCTION

Muscle contractions enhance neuronal adjustments regulated by activation of afferents originating from activated muscles. Indeed, among muscle afferents, metabosensitive fibers from groups III and IV are activated by metabolic, mechanical and thermal modification of their receptive fields occurring during and after repetitive contractions (Laurin et al., 2015). Metabolic agents such as lactic acid (LA) and potassium chloride (KCl), and electrically induced exercise (EIF) are also known to be specific activators of these thin metabosensitive afferents fibers (Rotto and Kaufman, 1988; Victor et al., 1988; Decherchi et al., 1998, 2001). When stimulated, these afferents change the motoneurons excitability in the spinal cord (Dousset et al., 2004; Laurin et al., 2010). Furthermore, these afferents are responsible for the sensation of muscle pain (Mense, 2009) and also project to brainstem level to induce an exercise pressor reflex (EPR), a neural drive originating from skeletal muscles that result in an increase in the sympathetic activity associated with an up-regulation of both heart and ventilation rate, and arterial blood pressure (McCloskey and Mitchell, 1972).

Neuromuscular system is highly malleable at young age, but this plasticity tends to be reduced at old age. Indeed, muscle strength progressively declines (Vandervoort and McComas, 1986) mostly because of sarcopenia, a phenomena resulting to a decrease in the number of type I and II muscle fibers, and to a type II muscle fiber atrophy (Lexell, 1995). Because muscle mass and phenotype were shown to take part in determining the magnitude of the EPR and the response of the metabosensitive afferents (Iwamoto and Botterman, 1985; Wilson et al., 1995; Xing et al., 2008; Caron et al., 2015), sarcopenia could be at the origin of a down regulation of the EPR and a decrease in the metabosensitive activity. This was suggested by some authors reporting a down regulation of the EPR with aging (Markel et al., 2003; Houssiere et al., 2006) but disputed by other showing that the EPR was maintained with aging (Ng et al., 1994; Greaney et al., 2013). Nevertheless, a recent study underlined an age-related alteration of the contribution of the metabosensitive muscle afferents to the hemodynamic response to exercise (Sidhu et al., 2015). Finally, because it was described that a period of inactivity induces a lower EPR during leg isometric exercise and during post-exercise ischemia (Kamiya et al., 2004) and an abnormal EPR in many forms of hypertension, heart failure or muscular dystrophy, exercise training has been proposed to restore the EPR (Murphy et al., 2011; Smith et al., 2014).

Thus, the aim of the present study was to record, with electrophysiological tools, the response of metabosensitive afferent fibers originating from *tibialis anterior* and *soleus* muscles to chemical injections of LA and KCl, EIF at 3, 6, 12 and 20 months of age in non-exercised rats, and at 12 and 20 months of age after 8 weeks of incremental treadmill running protocol. We hypothesized that metabosensitive afferents response is altered with aging and that repeated physical activity could reverse this alteration.

MATERIALS AND METHODS

Animals and Ethical Approval

Sixty-nine male Sprague Dawley rats (Janvier Lab®, France) were housed in smooth-bottomed plastic cages at 22°C in a room maintain on a 12-h light/dark cycle. Food (Safe®, France) and water were available *ad libitum*. Forty-eight were randomly allocated into 4 groups according to their age: 3 months (3 M, n = 12), 6 months (6 M, n = 12), 12 months (12 M, n = 12) and 20 months (20 M, n = 12), and allowed to age until electrophysiological recordings. Two other groups performed treadmill training during 8 weeks before the electrophysiological session: 12 months old (12M-EXE, n = 10) and 20 months old (20M-EXE, n = 11). The exercise effects in the 12M-EXE and 20M-EXE groups were compared to the 12 M and 20 M animals from the non-exercised groups, respectively.

All procedures outlined in this study were approved (license n°A 13.013.06) by the animal ethics committee of *Aix-Marseille University* (AMU) and *Centre National de la Recherche Scientifique* (CNRS). All experiments were performed in accordance with the recommendations listed in the Guide for Care and Use of Laboratory Animals (U.S. Department of Health and Human Services, National Institutes of Health) and the European Community's council directive of 24 November 1986 (86/609/ EEC).

Exercise Training Protocol

Rats were first familiarized with the treadmill for 1 week. Then, animals were trained 3 times per week on a treadmill with a progressive 8 weeks protocol inspired from Pasini et al. and previously described (Pasini et al., 2012; Caron et al., 2016). Briefly, duration of the exercise in the first and the second week was 10 and 20 min, respectively, with a running speed fixed at 13.5 m/min. For the next 3 weeks, running time and speed were progressively increased to reach at the 5th week a duration of 50 min at 15 m/min speed. Finally, for the last 3 weeks, exercise was performed for 60 min and speed was increased up to 18 m/min until the 8th week. All animals ran steadily on the treadmill. Rats were anesthetized for electrophysiological recordings within the 48h after the end of the exercise-training protocol.

Electrophysiological Recordings

When animals reach the age of their respective group, they were anesthetized with urethane (1.1 $g.kg^{-1}$ i.p.), and atropine (1 $mg.kg^{-1}$, i.p.) was administered to reduce airway secretions. The surgery and the afferent recordings were performed as previously described (Decherchi et al., 1998; Caron et al., 2014, 2015). Briefly, a catheter was inserted into the right femoral artery to let the blood flow freely to the left lower limb muscles and pushed up to the fork of the abdominal aorta in order to transport chemicals (i.e., potassium chloride [KCl] and lactic acid [LA]) to the controlateral muscle. Branches of nerves innervating the *soleus* and *tibialis anterior* muscles were dissected free from surrounding tissues, cut distally, immersed in paraffin oil and placed on two pairs of tungsten bipolar cuff electrodes for afferents recordings. The knee and ankle were firmly held by clamps on a horizontal support in order to avoid disturbing movements and to maintain the 90° knee joint angle during electrical muscle stimulation inducing an EIF. Animal temperature was maintained between 36 and 37°C with a blanket controlled by a rectal temperature probe.

Activities originating from the nerves were recorded and referred to a ground electrode implanted in a nearby muscle, amplified (1–100 K), filtered (30 Hz to 10 kHz) with a differential amplifier (P2MP® SARL, France) and fed into an amplitude window discriminators (P2MP® SARL) analyzing action potentials. The discriminators separated action potentials on the basis of their amplitude and provided an output pulse for the desired signal. For every waveform peak that appears within the window aperture (crossing the lower level of the windows) set by the user, a rectangular pulse was generated. Signals exceeding the upper level of the window (crossing the lower and upper levels) were not considered. Multiplexing the input signal and window discriminators provided convenient oscilloscope visualization and ease of setting up the experiment. It eliminated adjusting the oscilloscope levels for drift. In absence of any movement, only metabosensitive afferent fibers exhibiting spontaneous tonic low frequency baseline activity were active

(Decherchi et al., 1998). Thus, metabosensitives afferent activities were selected according to their action potential amplitudes which were higher than the background noise. The output provided noise-free tracings (discriminated units) on which action potentials were displayed on a computer and then counted using data analysis system (Biopac MP150® and AcqKnowledge® software, United States) at 1s intervals (in Hz). Baseline discharge was calculated during the 1-min period preceding injections or EIF, and its change was measured following specific activations. Afferent response variations were expressed in percentage of the corresponding baseline discharge rate ($F_{impulses}.s^{-1}$, % of baseline activity).

In a first step, distinct concentrations of KCl (1, 5, 10, and 20 mM / 0.5 ml) and LA (0.5, 1, 2, and 3 mM / 0.1 ml) were randomly injected into the catheter and were washed with 0.1 ml of normal saline. Each injection was separate by 10 min of recovery in order to let the afferent activity go back to its baseline activity.

In a second step, after a 30-min resting period, a 3-min EIF was performed. For this purpose, rhythmic muscle contractions were produced by a stimulator (Grass S88K®, United States) delivering rectangular pulse trains to a pair of steel electrodes placed on the muscle surface (pulse duration: 0.1 ms; frequency: 10 Hz, i.e., 5 shocks in each 500 ms train; duty cycle: 500/1000 ms). The voltage used was 20% higher than that used to elicit a maximal contraction.

At the end of the experiments, animals were sacrificed by an overdose (3 ml, i.a) of sodium pentobarbital solution (60 $mg.kg^{-1}$, Nembutal®, Sanofi Santé Animale, France).

Statistics

Data processing was performed using Sigmaplot® 14 SPSS. Data were expressed as mean ± SEM. Differences were tested by two-way analysis of variance (ANOVA test, factors: group x timing) completed by a Student-Newman-Keuls *post hoc* test to compare the metabosensive afferent responses to KCl and LA injections during aging process and after treadmill training (factors: age x doses). One-way ANOVA were used to compare EIF and muscle properties during aging process, while *t*-test were used to evaluate the differences after the training protocol.

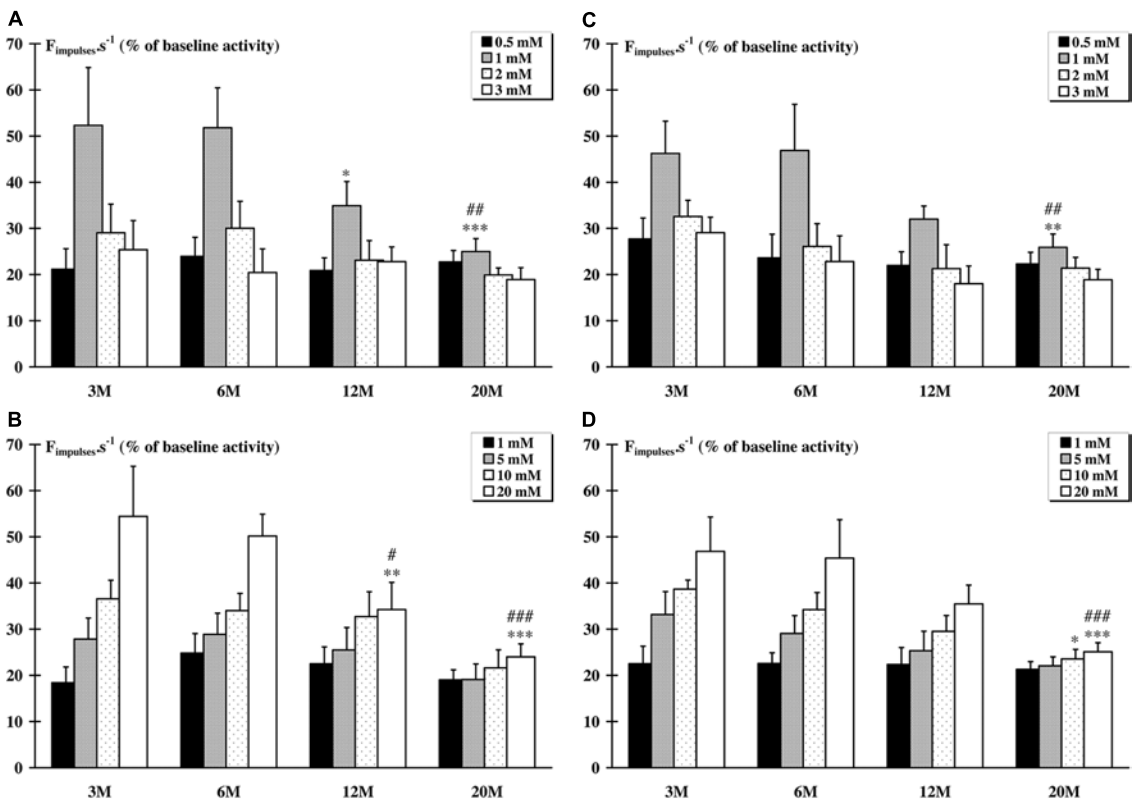

FIGURE 1 | Response of metabosensitive afferents fibers to chemical injections (LA and KCl). Whatever the dose injected, a significant ($p < 0.05$) increase in the raw afferent activity was recorded as compared to baseline activity in all groups. **(A)** The response of afferent fibers from the *tibialis anterior* muscle to 1 mM LA injection was significantly (*$p < 0.05$) lower in the 12 M group compared to the 3 M group, and in 20 M group compared to the 3 M (***$p < 0.001$) and to the 6 M (##$p < 0.01$) groups. **(B)** The response afferent fibers from the *tibialis anterior* muscle to 20 mM KCl injection was significantly lower in the 12 M group compared to the 3 M (**$p < 0.01$) and the 6 M (#$p < 0.05$) groups, and in the 20 M group compared to the 3 M (***$p < 0.001$) and to the 6 M (###$p < 0.001$) groups. **(C)** The response of afferent fibers from the *soleus* muscle to 1 mM LA injections was significantly lower in the 20M group compared to the 3 M (**$p < 0.01$) and the 6 M (##$p < 0.01$) groups. **(D)** The response of afferent fibers from the *soleus* muscle to 10 mM KCl injection was significantly ($p < 0.05$) lower in the 20 M group compared to the 3M group. Furthermore, the response to 20mM KCl injection was significantly lower in the 20 M group compared to the 3 M (***$p < 0.001$) and to the 6 M (###$p < 0.001$), and the response to 10 mM KCl injection is significantly lower (*$p = 0.015$) for the 20 M compared to 3 M group.

Results were considered statistically significant if the *p*-value fell below 0.05.

RESULTS

Afferents characterized as metabosensitive fibers exhibit spontaneous tonic low frequency baseline activity (4–10 Hz) under our experimental conditions. Whatever the dose and the stimulus used (LA or KCl and EIF), a significant ($p < 0.05$) increase in the raw afferent activity was recorded as compared to baseline activity within each type of muscle and experimental groups. In this experiment, in accordance with previous ones performed in the *tibialis anterior* and *soleus* muscles of Sprague-Dawley rats, we observed that the activation of muscle afferents by LA culminated for the 1 mM concentration and then declined whereas there was a relationship between the doses of KCl and the change in afferent discharge rate (Decherchi et al., 1998; Martin et al., 2009; Caron et al., 2014, 2015).

Response to LA and KCl Injections

The pattern of responses of metabosensitive muscle afferents activated by increased interstitial concentrations of LA or KCl consisted of a burst of activity beginning within 5–10 s after the bolus injection. Recovery of baseline spontaneous discharge rate value always occurred within 3 min. For the *tibialis anterior* muscle, the responses to LA injections were decreased only for the concentration of 1 mM in animals aged of 12 ($p < 0.05$) and 20 ($p < 0.001$) months, compared to animals aged of 3 months (**Figure 1A**). The response to KCl injections was also decreased for the highest concentration of 20 Mm in the 12 M ($p < 0.01$) and 20 M ($p < 0.001$) groups (**Figure 1B**). For the *soleus* muscle, only the response to 1 mM LA concentration was decreased ($p < 0.01$) in animals from the 20 M group (**Figure 1C**). The response to KCl injections was also decreased for the 10 mM ($p < 0.05$) and 20 mM ($p < 0.001$) concentration in the 20 M group (**Figure 1D**).

Exercise training did not change the responses to KCl and LA injections for both muscles compared to the corresponding non-exercised animals (**Figure 2**).

Response to EIF

An alteration of the response to EIF was observed with aging. Indeed, a significant ($p < 0.05$) lower afferent discharge following a 3-min EIF was observed in the 20 M groups for both muscles (**Figure 3A**). However, physical activity did not induce notable changes in the afferents response to EIF in aged animals although, for the both *tibialis anterior* and *soleus* muscles, there is a

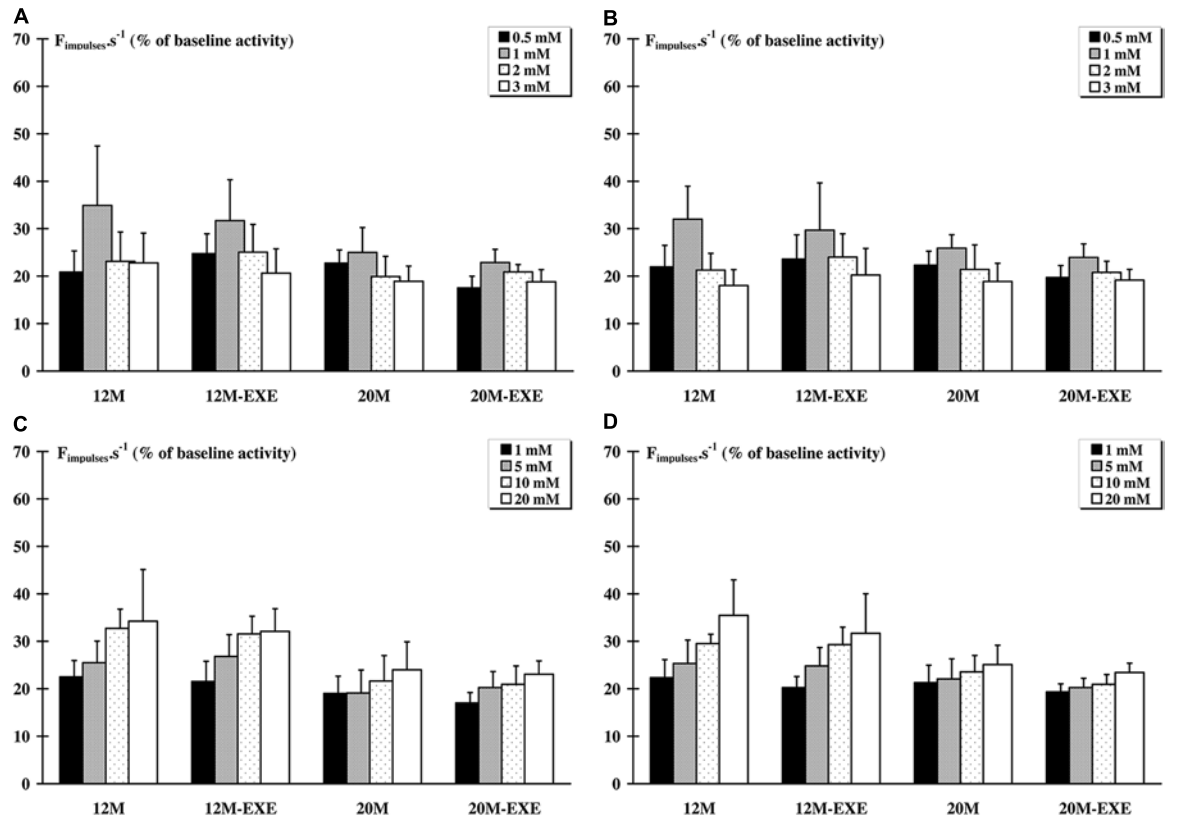

FIGURE 2 | Comparison of the response of metabosensitive afferents fibers to chemical injections (LA and KCl) between non-active and active aged rats. Whatever the dose injected, a significant ($p < 0.05$) increase in the raw afferent activity was recorded as compared to baseline activity in all groups. The response of afferent fibers from *tibialis anterior* muscle to LA **(A)** and KCl **(C)** injections did not differ between old non-exercised and old trained animals. The response of *soleus* muscle afferents to LA **(B)** and to KCl **(D)** injections also did not show any difference between old non-exercised and old exercised animals.

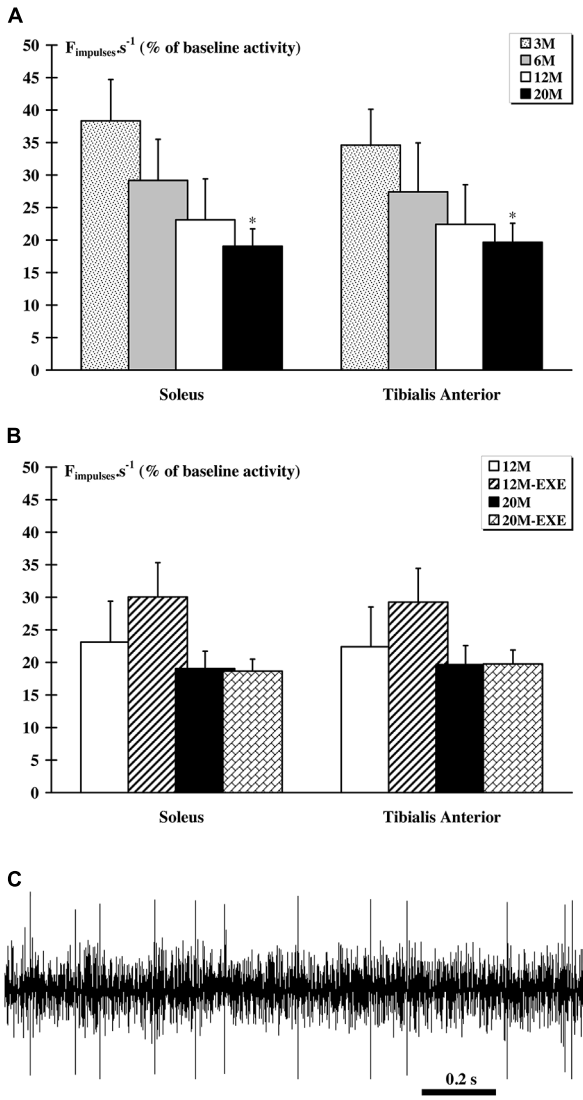

FIGURE 3 | Comparison of the response of metabosensitive afferents fibers to muscle electrically-induced fatigue (EIF) between non-active and active aged rats. In all groups, a significant ($p < 0.05$) increase in activity was recorded after 3-min EIF. **(A)** In sedentary groups, the magnitude of the response to EIF decrease with age and was significantly ($p < 0.05$) lower in the 20 M group compared to the 3 M group whatever the muscle considered. **(B)** In the older animals, whatever the muscle considered and despite an increasing tendency for 12M-EXE group, no difference was noted. **(C)**. Example of extracellular recording of afferent fibers from *tibialis anterior* muscle in 12 M-EXE group.

tendency to increase for 12M-EXE groups (**Figure 3B**). An example of raw recording obtained in the 12M-EXE group is showed in **Figure 3C**.

DISCUSSION

Our study reported that the response of metabosensitive afferent fibers from *tibialis anterior* and *soleus* muscles decreased with age for the doses of KCl and LA inducing the highest responses. Namely, from age of 12 months for the *tibialis anterior* muscle and 20 months for the *soleus* muscle, the responses to the dose of KCl (20 mM) and LA (1 mM) that induced the highest afferents response were reduced. Furthermore, the response to EIF was reduced for the *soleus* muscle at age of 20 months. Our results also indicated that 8 weeks of incremental treadmill running exercise did not reverse these alterations observed at 12 or 20 months.

Effect of Aging on Metabosensitive Response.

Considering that the afferent discharge depends of the amount of metabolites released during EIF, of the number of receptors (TRPV1, ASIC3 and P2X) of terminals endings binding the metabolites endogenously released or exogenously injected, and of the number of afferents fibers on the nerve (Gao et al., 2006, 2007; Light et al., 2008), any change in any of these elements may affect the recorded response. However, it is difficult to determine the cause of this diminished response although some hypotheses can be advanced.

Anatomical studies have indicated that aging may be associated to a loss of thin myelinated and unmyelinated afferents fibers (Ceballos et al., 1999). This loss could start in the tibial nerve at 12 months of age (−59% of thin myelinated and −15% of unmyelinated fibers) and could reach 50% of fiber loss in very old animals (33 months) (Ceballos et al., 1999). However, other study founded no significant difference in the total number of neurons in lumbar dorsal root ganglia (DRG) between young and old animals (30 months) (Bergman and Ulfhake, 1998). Finally, electrophysiological study in human indicated that aging is also associated with a relative atypical unmyelinated C-fiber increase (Namer, 2010). In view to these studies, it is difficult to assume that the reduction of the number of thin myelinated and unmyelinated metabosensitive afferent fibers during aging could be responsible for the deterioration of their response.

Another explanation could be found in the receptors on the surface of the metabosensitive terminations. Wang et al. (2006) reported a decline in TRPV1 (transient receptor potential vanilloid type 1) expression in DRG and in nerve afferents innervating hind-limbs with aging but no change in TRPV1 mRNA level indicating a lower protein expression. Reduced levels of TRPV1 were also found in tibial nerve of aged animals, suggesting that TRPV1 transport is less efficient when getting older. TRPV1 level is suggested to be modulated by trophic factors, especially artemin, which receptors (GFRα3 or GDNF family receptors alpha-3) are highly co-localized with TRPV1 (Orozco et al., 2001; Elitt et al., 2006). In aged animals, because it was reported a concomitant decreased level of TRPV1 and GFRα3, and a decreased trophic support in the DRG (Wang et al., 2006), we can assume that the decrease of growth factors with aging may reduce the number of TRPV1 in metabosensitive nerve endings and consequently their responses when stimulated by metabolic agents released by muscles. However, a study indicated that the numbers of discharges induced by low pH, ATP, bradykinin, cold and heat stimuli were not different with aging (Taguchi and Mizumura, 2011).

It was reported that the conduction velocity of myelinated fibers decreases about 10–15% with age in nerve innervating the *gastrocnemius* and *soleus* muscles and in vagus nerve but not in unmyelinated fibers (Sato et al., 1985). Because metabosensitive afferents are composed of thinly myelinated and unmyelinated fibers, any change in the myelin sheath will affect the response of these afferents in a lesser extent.

Finally, because the EIF response results in metabosensitive afferent activation following muscle metabolite production, any decrease in metabolite production should affect their response. In our study, the EIF response was significantly reduced in metabosensitive afferents from *soleus* and *gastrocnemius* muscles at age of 20 months. This result suggests that the production of metabolites (lactate, K^+, inflammatory mediators) by fatigued muscle could be affected with age. The literature described muscle anatomical changes during aging process. Indeed, aging is characterized by a progressive loss in muscle mass and a decrease number of type I (slow) and II (fast) muscle cells (muscle cell apoptosis), associated with type II cell atrophy (Lexell et al., 1988; Lexell, 1995; Narici et al., 2003). Because the metabosensitive response depends, among others, of the muscle mass and muscle phenotype (Caron et al., 2015), any change in muscle architecture may alter the release of metabolites during fatigue and consequently the afferent response to EIF.

Effect of Exercise

Many studies show the benefit of physical exercise on pathologies or during aging. For example, it was shown the beneficial effect of aerobic exercise training on neuropathic pain (Dobson et al., 2014). It was also shown that exercise can reduce hyperglycemia and the risk to develop diabetes associated illness (Balducci et al., 2010; Li and Hondzinski, 2012), or can help to recover from peripheral nerve injury (Marqueste et al., 2004; Keeler et al., 2012; Wilhelm et al., 2012). As previously mentioned exercise training can also be an effective strategy to normalize the EPR in case of hypertension, heart failure or muscular dystrophy (Murphy et al., 2011; Smith et al., 2014). Finally, the literature reports that exercise training partially prevented the decreased of TRPV1 in DRG afferents in rats with chronic heart failure (Wang et al., 2012). Moreover, physical activity has been shown to maintain normal levels of artemin and GDNF after spinal cord injury (Detloff et al., 2014).

Assuming that alteration of the metabosensitive response with aging (20 M group) is due to a decrease in TRPV1 level and that our daily incremental treadmill exercise protocol during the last 8 weeks preceding the recordings may maintain normal level of TRPV1, we should have observed a restored response in the 12M-EXE and 20M-EXE groups. This has not been the case; the responses to KCl and LA injections, and to EIF were similar in exercised animals (12M-EXE and 20M-EXE groups) compared to non-exercised animals (12 M and 20 M groups). Only in group 12M-EXE, we observed a response that tended to increase in response to EIF. However, because of the large variability among animals, this increase was not significant. The lack of significant results could be due to the duration of the exercise we chose. Indeed, it was previously shown in rodents that a 6-month duration of aerobic exercise induced a better neuroprotection in mice model of Alzheimer disease (Garcia-Mesa et al., 2011). The authors showed that this long-lasting exercise induced benefits on synapse, redox homeostasis and general brain function. Even if our exercise protocol during the last 8 weeks preceding the recordings was not detrimental in older rats, one could hypothesize that a longer duration of exercise over months could have led to maintain an efficient afferent activity from trained muscles.

CONCLUSION

Our study showed that the metabosensitive responses to metabolite injections and EIF were altered with aging and that a daily incremental treadmill exercise protocol during the last 8 weeks preceding the recordings does not restore these responses. Because these fibers are involved in regulation of sensorimotor loop, muscle pain sensation and EPR, and in physiological adjustments (Mitchell et al., 1977; Mazzone and Geraghty, 1999; Coull et al., 2003; Decherchi and Dousset, 2003; Edwards et al., 2003; Decherchi et al., 2004, 2007; Cole et al., 2010), their alteration may be responsible of some troubles observed with aging during walking and running (Markel et al., 2003; Houssiere et al., 2006), and at rest (Ng et al., 1994; Markel et al., 2003). If a repeated exercise performed when adult does not seem to reverse the effects of aging on metabosensitive afferents, it has been proved that exercise induces many positive outputs on neuromuscular functions (Koceja et al., 2004). In the future, it would be interesting to compare the type of exercise we have chosen in this study to others types of exercise or to animals that have been performed repeated exercises since they were young (i.e., throughout life).

AUTHOR CONTRIBUTIONS

GC, PD, and TM designed the study, carried out the analysis, analyzed the data, wrote the manuscript, and gave the final approval for publication.

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The Effects of Physical Training on Quality of Life, Aerobic Capacity, and Cardiac Function in Older Patients With Heart Failure: A Meta-Analysis

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Aim: The purposes of this meta-analysis were to quantify the effectiveness of physical training on quality of life (QoL), aerobic capacity, and cardiac functioning in older patients with heart failure (HF) and evaluate dose–response relationships of training variables (frequency, volume, and duration).

Methods: Scholarly databases (e.g., PubMed/MEDLINE, Google Scholar, and Scopus) were searched, identifying randomized controlled trials that investigated the effectiveness of different training modes on QoL (assessed by the Minnesota Living with Heart Failure Questionnaire), aerobic capacity (assessed by the 6 min walk test) and cardiac function (assessed by left ventricular ejection fraction).

Results: Twenty five studies were included with a total of 2,409 patients. Results showed that exercise training improved total QoL (small ES = −0.69; 95% CI −1.00 to 0.38; $p < 0.001$), aerobic capacity (small ES = 0.47; 95% CI 0.15–0.71; $p = 0.002$) and cardiac function (moderate ES = 0.91; 95% CI 0.37–1.45; $p = 0.001$). In addition, univariate analyses revealed the moderating variable 'training mode' significantly influenced aerobic capacity ($Q = 9.97$; $p = 0.007$), whereby, resistance training had the greatest effect (ES = 1.71; 95% CI 1.03–2.39; $p < 0.001$), followed by aerobic training (ES = 0.51; 95% CI 0.30–0.72; $p < 0.001$), and combined training (ES = 0.15; 95% CI −0.24 to 0.53; $p = 0.45$). Meta-regression analysis showed that only the duration of an intervention predicted the effect of physical training on QoL (coefficient = −0.027; $p = 0.006$), with shorter training durations (12 weeks) showing larger improvements.

Conclusion: The present meta-analysis showed that physical training has positive effects on QoL, aerobic capacity, and cardiac function in older patients with HF. Practitioners should consider both training volume and mode when designing physical training programs in order to improve QoL and aerobic capacity in older patients with HF.

Keywords: exercise training, resistance training, physical function, health status, cardiac rehabilitation

INTRODUCTION

Heart failure (HF), a complex clinical syndrome characterized by reduced ability of the heart to pump and/or fill with blood, represents a major public health problem, with a computed prevalence of over 5.8 million in the USA, and over 23–26 million worldwide (Roger, 2013). Being a global pandemic, prevalence is still increasing and is expected to reach 8 million people in the USA by 2030, whereas up to 15 million people are living with HF in Europe (Maggioni, 2015). In Italy, the burden is especially prevalent among elderly people and in Regions such as Liguria, which is a Region with Europe's oldest residents (Marangoni et al., 2012).

As such, HF imposes high societal costs and impacts on patient quality of life (QoL) (Heo et al., 2009). QoL relates to a multitude of aspects such as physical fitness, social environment, education, employment, economic and finance conditions, and other markers such as religion and beliefs, and environment (Katschnig, 1997; Coelho et al., 2005). Among these elements, physical and mental health play an important role in management and characterization of QoL of a person (Rodríguez-Fernández et al., 2017), and especially a patient with cardiac disease (Pedersen et al., 2007). In fact, it has been shown that low pre-hospitalization health-related QoL and poor fitness are associated with lower post-hospitalization general health, increased remission rates, and mortality risk (Mendes de Leon et al., 1998; Hawkes et al., 2013).

Prevention of cardiac disease based on physical activity plays a major role in public health, and physical activity should be considered a priority in prevention of chronic-degenerative disorders (Romano-Spica et al., 2015). Regular physical activity mitigates the risk of HF hospitalization and mortality (Hegde et al., 2017). There seems to be a strong, dose-dependent association between physical

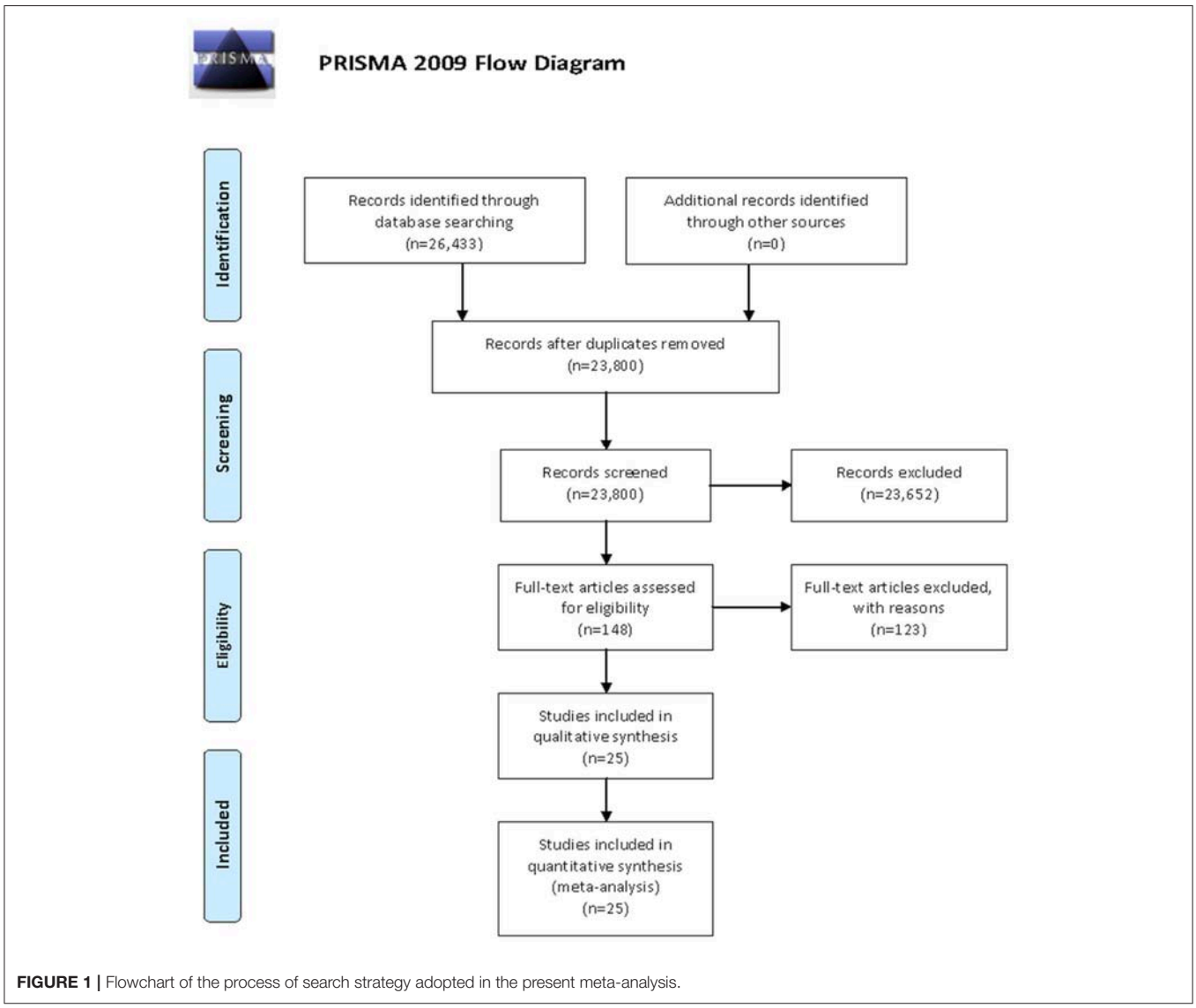

FIGURE 1 | Flowchart of the process of search strategy adopted in the present meta-analysis.

activity/fitness and the risk of HF. As such, exercise can be conceived as a non-pharmacological treatment to counter the pathophysiological mechanisms leading to HF (Pandey et al., 2015).

Physical activity generally improves QoL, aerobic fitness, and cardiac function in HF patients (Beniaminovitz et al., 2002; Harris et al., 2003; Gary et al., 2004; Koukouvou et al., 2004; Davidson et al., 2010; Hassanpour Dehkordi and Khaledi Far, 2015). However, this is not always the case, as Keteyian et al. (1999) showed no significant change in QoL after 6 months' aerobic training in male HF patients.

Different modes of training (namely aerobic, resistance, and combined training) result in divergent physiological adaptations, and the most beneficial mode for HF patients remains unclear. Delagardelle et al. (2002) and Mandic et al. (2009) reported that combined aerobic and resistance training was superior to aerobic training exclusively for improving cardiorespiratory fitness (i.e., peak oxygen uptake [VO_2peak]). In contrast, Haykowsky et al. (2005) reported no significant difference between combined aerobic and resistance training and aerobic training only in cardiorespiratory fitness (i.e., VO_2peak), and therefore, ambiguity remains as to which modality of training is optimal for patients with HF. Moreover, most investigations report no difference between short- and long-term training for QoL and cardiorespiratory fitness (i.e., 6 min walk test [6-MWT]) improvement (Dracup et al., 2007; Jolly et al., 2009; Davidson et al., 2010).

Previous meta-analyses have shown exercise training improves cardiorespiratory fitness, ejection fraction and QoL in cardiac patients (Piepoli et al., 2004; Smart and Marwick, 2004; Haykowsky et al., 2007; Giuliano et al., 2017; Ostman et al., 2017). More specifically, Haykowsky et al. (2007) reported that aerobic training improved ejection fraction in patients with HF to a greater extent than combined aerobic and strength training. Furthermore, Ostman et al. (2017) reported that high-intensity physical training reduced total QoL score (i.e., improved QoL). However, despite the existing meta-analyses in the field, whether there is a most effective training mode and/or dose-response relationship remains unknown. The effectiveness of physical training on QoL, aerobic capacity, and left ventricular ejection fraction in older HF patients in terms of training variables (frequency, volume, and duration) is still unknown. This knowledge would allow practitioners to (i) maximize training-related health benefits and athletic performance and (ii) adequately design strength and conditioning programs for cardiac rehabilitation.

Therefore, the present meta-analysis was designed in order to fill the aforementioned void in knowledge. In particular, the aim of this meta-analysis was to establish the effects of physical training on QoL, aerobic capacity, and left ventricular ejection fraction in older HF patients. A secondary aim was to quantify dose-response relationships according to training modalities and program variables.

MATERIALS AND METHODS

Search Strategy

This meta-analysis was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines (**Figure 1**, Moher et al., 2009). A systematic literature search was conducted for randomized controlled trials (RCTs) studying the effects of physical training on QoL in older patients with HF. Studies were obtained through systematic manual and electronic searches (up to May 1st, 2018) in electronic databases (i.e., Google Scholar, MEDLINE/PubMed, and Scopus). Electronic databases were searched using the following search syntax with keywords and/or MeSH terms: [("aerobic training" OR "resistance training" OR "power training" OR "plyometric training" OR "exercise") AND ("elderly" OR "older") AND "heart failure" AND ("quality of life" OR "Minnesota Living with Heart Failure Questionnaire" OR "walking test" OR "left ventricular ejection fraction")]. Moreover, we performed manual searches of relevant journals and reference lists obtained from published articles. The present meta-analysis included studies published in journals that reported original research data from older patients with HF.

Inclusion and Exclusion Criteria

Studies were included in this meta-analysis if they met all the following Population/Intervention/Comparison/Outcome(s) (PICOS) criteria:

(1) Population: studies recruiting older patients with HF as participants from different countries (i.e., Belgium, Brazil, Canada, Greece, Iran, Italy, Netherlands, Sweden, Taiwan, United States, United Kingdom); Older patient groups includes the younger old (65–74 years), the old (75–84 years), and the older old or oldest old (>85 years) (Little et al., 2012). However, we allowed 50 years of age as a minimum reference range of age for studies on African population (World Health Organization, 2016), and from 65 years for populations from developed countries (Kowal and Dowd, 2001).
(2) Intervention or exposure:
    a) Studies examining the effects of physical training on QoL, aerobic capacity, and cardiac function in older patients with HF;
    b) Studies describing their training variables (e.g., volume, frequency, and duration).
(1) Comparison: Studies involving a control group against which an intervention was compared.
(2) Outcome(s): QoL, aerobic capacity, or cardiac function assessed using the Minnesota Living with Heart Failure Questionnaire (MLWHFQ), the 6-MWT (i.e., total distance covered), and left ventricular ejection fraction, respectively. In addition, we examined how moderating variables like training duration (weeks), training frequency (sessions/week), and type of training, influenced physical training related QoL, aerobic capacity and cardiac function enhancements.
(3) Study design: original research in the form of RCTs.

TABLE 1a | Effect of physical training on quality of life.

Study	Sex	Training mode	Training period (weeks)	Session duration (min)	Number of sessions per week	Exercise group						Control group					
						Before			After			Before			After		
						N	Mean	SD	*N*	Mean	SD	*N*	Mean	SD	*N*	Mean	SD
Beniaminovitz et al., 2002	Both	Combined	4	30	3	17	50	3.8	17	37	3	8	41	4.6	8	33	3.5
Harris et al., 2003	Both	Resistance	6	30	5	24	36.3	4.21	24	31	3.66	22	28.7	4.7	22	25.5	4.61
Chien et al., 2011	Both	Combined	8	30	3	24	11	11	22	7	9	27	16	16	22	13	13
Davidson et al., 2010	Both	Aerobic	16	60	1	53	44.11	23.7	50	27.9	12.46	52	52.37	26.38	44	36.89	16.22
Kitzman et al., 2013	Both	Aerobic	16	60	3	24	36	19	24	26	19	30	28	23	30	25	22
Kitzman et al., 2010	Both	Aerobic	16	60	3	26	32	20	26	25	24	27	25	22	27	27	19
Edelmann et al., 2011	Both	Combined	16	30	2–3	44	14	14	44	9	8	20	13	0	20	11	9
Brubaker et al., 2009	Both	Aerobic	16	60	3	30	39.9	4.2	30	25.3	4.2	29	44.1	4.9	29	37.9	4.2
Chrysohoou et al., 2014	Both	Aerobic	12	45	3	33	21	7	33	7	9	39	19	12	39	21	13
Dracup et al., 2007	Both	Combined	12	45	4	86	46.7	23.8	86	37.5	23.9	87	49.2	22.4	87	46.7	26.5
McKelvie et al., 2002	Both	Combined	12	30	2–3	91	32.5	2.5	70	28.6	1.9	90	28.6	2.1	73	27.4	1.5
Gary and Lee, 2007	Female	Aerobic	12	25		13	38	26	13	20	16	10	24	16	10	25	18
Fu et al., 2016	Both	Aerobic	12	32.5	3	30	41	2.1	30	22.5	2.3	30	42	2.6	30	40.1	2.8
Fu et al., 2016	Both	Aerobic	12	32.5	3	30	42.1	2.5	30	24.1	3.1	30	41.3	2.3	30	41.8	2.8
Mandic et al., 2009	Both	Aerobic	12	30	3	14	45.9	16.8	14	41.4	23.2	13	40.2	22.5	13	37.8	24.7
Mandic et al., 2009	Both	Combined	12		3	15	40	19.8	15	32.6	20.2	13	40.2	22.5	13	37.8	24.7
Patwala et al., 2009	Both	Aerobic	12	30	3	25	34.6	22.3	25	26.2	20.5	25	29	16.3	25	29.5	17.8
Maria Sarullo et al., 2006	Both	Aerobic	12	30	3	30	31.2	2.2	30	35.5	1.9	30	30.6	2.5	30	31.2	2.6
Servantes et al., 2012	Both	Aerobic	12	57.5	3–4	17	40.4	17.9	17	20.7	16.3	11	46.5	18.5	11	51	16.8
Servantes et al., 2012	Both	Combined	12		3–4	17	45.1	20.8	17	25.1	16.5	11	46.5	18.5	11	51	16.8
van den Berg-Emons et al., 2004	Both	Aerobic	12	60	2	18	24.1	19.7	18	18.1	18.5	16	27.5	13.9	16	26.5	12.7
Davidson et al., 2010	Both	Aerobic	54	60	1	53	44.11	23.7	50	52.9	15.68	52	52.37	26.38	42	56.43	18.28
Jolly et al., 2009	Both	Combined	54	25	5	84	33.35	21.3	84	37.61	20.97	85	31.82	22.5	85	34.91	24.8
McKelvie et al., 2002	Both	Combined	54	30	2–3	91	32.5	2.5	57	29.1	2.4	90	28.6	2.1	67	25.3	1.7
Pihl et al., 2011	Both	Resistance	54	45	3–4	28	22.4	15.5	28	25.2	10	31	24	17.1	31	25.3	11
Conraads et al., 2007	Both	Aerobic	20	60	3	8	51	9	8	30	6	9	36	8	9	24	7
Hassanpour Dehkordi and Khaledi Far, 2015	Both	Aerobic	24	40	3	30	65.12	15.93	30	70.28	19.33	31	64.85	17	31	57.92	18.5
Dracup et al., 2007	Both	Combined	24	45	4	86	46.7	23.8	86	35.7	23.7	87	49.2	22.4	87	43.2	27.3
Evangelista et al., 2017	Both	Combined	24	27.5	5	19	48	18.1	19	39.1	20.4	27	46.9	21	27	49.4	25
Evangelista et al., 2017	Both	Combined	24	27.5	5	16	43.9	22.9	16	36.7	21.7	27	46.9	21	27	49.4	25
Evangelista et al., 2017	Both	Combined	24	27.5	5	9	45.5	22.9	9	47.7	20.9	27	46.9	21	27	49.4	25
Jolly et al., 2009	Both	Combined	24	25	5	84	33.35	21.3	84	36.26	24.08	85	31.82	22.5	85	34.49	23.98
Keteyian et al., 1999	Male	Aerobic	24	33	3	21	38	28	21	31	22	22	28	18	22	26	23
Koukouvou et al., 2004	Male	Aerobic	24	60	3–4	16	45.5	17.1	16	34.1	13	10	45.1	9.9	10	45.2	9

TABLE 1b | Effect of physical training on aerobic capacity (i.e., 6 min walk test).

Study	Sex	Training mode	Training period (weeks)	Session duration (min)	Number of sessions per week	Exercise group						Control group					
						Before			After			Before			After		
						N	Mean	SD	*N*	Mean	SD	*N*	Mean	SD	*N*	Mean	SD
Harris et al., 2003	Both	Resistance	6	30	5	24	459	24	24	540	23	22	491	26	22	531	25
Chien et al., 2011	Both	Combined	8	30	3	24	424	145	22	433	145	27	432	81	22	429	93
Davidson et al., 2010	Both	Aerobic	16	60	1	53	279.45	110.97	50	361.2	132.34	52	251.06	112.57	42	274.98	106.6
Kitzman et al., 2013	Both	Aerobic	16	60	3	24	447	107	24	486	89	30	438	79	30	448	70
Edelmann et al., 2011	Both	Combined	16	30	2–3	44	545	86	44	569	88	20	551	86	20	568	80
Chrysohoou et al., 2014	Both	Aerobic	12	45	3	33	422	77	33	476	82	39	406	64	39	423	65
McKelvie et al., 2002	Both	Combined	12	30	2–3	91	434	7	70	456	12	90	421	8	73	436	13
Gary et al., 2004	Female	Aerobic	12	25		13	914	362	13	923	346	10	817	422	10	811	391
van den Berg-Emons et al., 2004	Both	Aerobic	12	60	2	18	455	71	18	501	96	16	435	77	16	448	84
Davidson et al., 2010	Both	Aerobic	54	60	1	53	279.45	110.97	47	386.55	129.97	52	251.06	112.57	33	247.27	122.96
McKelvie et al., 2002	Both	Combined	54	30	2–3	91	434	7	70	451	15	90	421	8	67	441	17

TABLE 1c | Effect of physical training on cardiac function (Left ventricular ejection fraction [%]).

Study	Sex	Training mode	Training period (weeks)	Session duration (min)	Number of sessions per week	Exercise group						Control group					
						Before			After			Before			After		
						N	Mean	SD	*N*	Mean	SD	*N*	Mean	SD	*N*	Mean	SD
Kitzman et al., 2013	Both	Aerobic	16	60	3	24	58	6	24	58	6	30	56	5	30	56	5
Kitzman et al., 2010	Both	Aerobic	16	60	3	26	61	5	26	57	8	27	60	10	27	55	8
Edelmann et al., 2011	Both	Combined	16	30	2–3	44	67	7	44	66	6	20	66	7	20	67	8
Brubaker et al., 2009	Both	Aerobic	16	60	3	30	31.1	2.2	30	29.4	1.8	29	32.8	2.4	29	29.1	2.3
McKelvie et al., 2002	Both	Combined	12	30	2–3	91	28.2	0.8	80	28.5	1.5	90	27.7	0.9	81	29.3	1.6
Fu et al., 2016	Both	Aerobic	12	32.5	3	30	57.6	1.9	30	57.8	1.7	30	56.5	2.2	30	54.4	3.3
Fu et al., 2016	Both	Aerobic	12	32.5	3	30	28.1	1.1	30	46.2	2.5	30	28.1	1.4	30	28	1.2
Mandic et al., 2009	Both	Aerobic	12	30	3	7	30.9	12.2	7	33.2	12.6	8	28.9	11.9	8	28.4	9.2
Mandic et al., 2009	Both	Combined	12		3	15	32.7	14.7	15	35.4	10.1	13	28.9	11.9	8	28.4	9.2
Patwala et al., 2009	Both	Aerobic	12	30	3	25	32.8	6.2	25	37.3	5.4	25	32.6	7	25	35	7.2
Maria Sarullo et al., 2006	Both	Aerobic	12	30	3	30	29.2	5	30	30.1	4	30	28.9	4	30	27.3	4
Conraads et al., 2007	Both	Aerobic	20	60	3	8	27	5	8	36	5	9	28	5	9	34	6
Hassanpour Dehkordi and Khaledi Far, 2015	Both	Aerobic	24	40	3	30	32	4	30	37	5	31	33	5	31	31	5

Studies were excluded if:

(i) They were reviews, opinion papers and commentaries, interviews, letters to the editor, editorials, posters, conference papers, abstracts, book chapters, or books. However, published review articles were examined to avoid missing relevant articles;

(ii) They did not include sufficient data to calculate standardized mean differences.

Coding of Studies

Two authors independently extracted data using a structured form. Because of the high number of variables that may affect training effectiveness, independent variables were grouped into the following areas as reported in the included studies: (i) training modes (aerobic vs. resistance vs. combined aerobic and resistance) and (ii) training variables (training duration in weeks [4–8 weeks vs. 12 weeks vs. 16 weeks vs. 5–6 months vs. 1 year], weekly training frequency [1–2 vs. 3 vs. 4 vs. 5 or more sessions per week], and session duration (20–30 min vs. 31–45 min vs. 46–65 or more min).

Data Extraction

The main study characteristics (i.e., intervention program, training variables, relevant outcomes) were extracted into a Microsoft Excel/spreadsheet.

Statistical Analyses

Data were extracted from the included studies using a standardized documentation form. Effect size (ES) and 95% confidence intervals (95% CI) were calculated for the identified studies. Meta-analyses were computed using the program Comprehensive Meta-Analysis, version 2 (Borenstein et al., 2005). Statistical heterogeneity was assessed using Q and I^2. The I^2 measure of inconsistency was used to examine between-study variability. Values of 25, 50, and 75% represent low, moderate, and high statistical heterogeneity, respectively (Higgins et al., 2003). Due to study heterogeneity, we applied a random-effects model for all comparisons. Potential publication bias was visually inspected with a funnel plot, looking at asymmetry of the graph. In addition, meta-regression analyses (method of moments) were applied to compute possible predictors that may have influenced training-related effects (e.g., training duration, weekly training frequency, and session duration). Effect sizes (ES) were classified as trivial (<0.35), small (0.35–0.80), moderate (0.80–1.50), or large (>1.5) (Rhea et al., 2003) and significance level was set *a priori* at $p < 0.05$.

RESULTS

Search Results

The applied search strategy yielded a preliminary number of 26,433 studies. After removing duplicates, 23,800 unique studies were screened. Screening of titles and abstracts resulted in 23,652 papers being discarded. This was due to the nature itself of the search strategy, which was designed to be the broadest possible in order to capture all relevant studies and was performed utilizing different scholarly databases, including the gray literature. Full texts of 148 articles were retrieved and assessed using the predetermined inclusion and exclusion criteria. After a careful review of full texts, 123 articles were excluded and the remaining 25 articles were included in this meta-analysis. A flow chart of the systematic search process is illustrated in **Figure 1**. Details of all included studies are depicted in **Tables 1A–C.**

Effects of Physical Training on Quality of Life in Patients With Heart Failure

Twenty five studies totalizing 34 ES were identified and a small effect of physical training on QoL was observed (ES = −0.69; 95% CI = −1.00 to −0.38; $p < 0.001$) (**Figure 2**). High heterogeneity was observed ($I^2 = 91.45\%$; $p < 0.001$). Therefore, sub-group analysis was conducted, observing a moderate QoL improvement in females (ES = −1.13; 95% CI = −2.01 to −0.24; $p = 0.013$), small QoL improvement in males (ES = −0.55; 95% CI = −1.29 to 0.19; $p = 0.148$), and small QoL improvement in males and females combined (ES = −0.69; 95% CI −1.02 to −0.36; $p < 0.001$), without significant difference between them ($Q = 1.05$; $p = 0.592$). When the effects of different intervention types were analyzed, QoL improvements were observed following aerobic training (moderate ES = −1.04; 95% CI = −1.67 to −0.41; $p = 0.001$) and combined aerobic and resistance training (small ES = −0.42; 95% CI −0.71 to −0.13; $p = 0.005$), with trivial effects after resistance training (ES = −0.17; 95% CI = −0.80 to 0.47; $p = 0.610$). However, no significant difference was observed between training modes ($Q = 4.20$; $p = 0.123$) (**Table 2**).

Effects of Physical Training on Aerobic Capacity and Cardiac Function in Older Patients With Heart Failure

Eleven studies examined the effects of physical training on aerobic capacity (i.e., total distance covered in the 6-MWT). The grouped effect (i.e., all type of physical training combined) revealed improvements following intervention (small ES = 0.43; 95% CI = 0.15 to 0.71; $p = 0.002$) (**Figure 3**). Small improvements were observed in males and females combined (ES = 0.46; 95% CI = 0.17–0.75; $p = 0.002$), with trivial effect on females (ES = 0.04; 95% CI = −0.78 to 0.87; $p = 0.922$). Due to insufficient data, the effect on males was not calculated. When different types of physical training were analyzed, large improvements were observed following resistance training (ES = 1.71; 95% CI = 1.03 to 2.39; $p < 0.001$), small improvements were observed following aerobic training (ES = 0.51; 95% CI = 0.30 to 0.72; $p < 0.001$), and trivial improvements were observed following combined aerobic and resistance training (ES = 0.15; 95% CI = −0.24 to 0.53; $p = 0.458$). Additionally, significant difference was observed between different training modes ($Q = 15.54$; $p < 0.001$) (**Table 2**).

Cardiac function (i.e., left ventricular ejection fraction) showed moderate improvements after physical training (moderate ES = 0.91; 95% CI = 0.37 to 1.45; $p = 0.001$) (**Figure 4**). When different interventions were analyzed, there were improvements following aerobic training (moderate

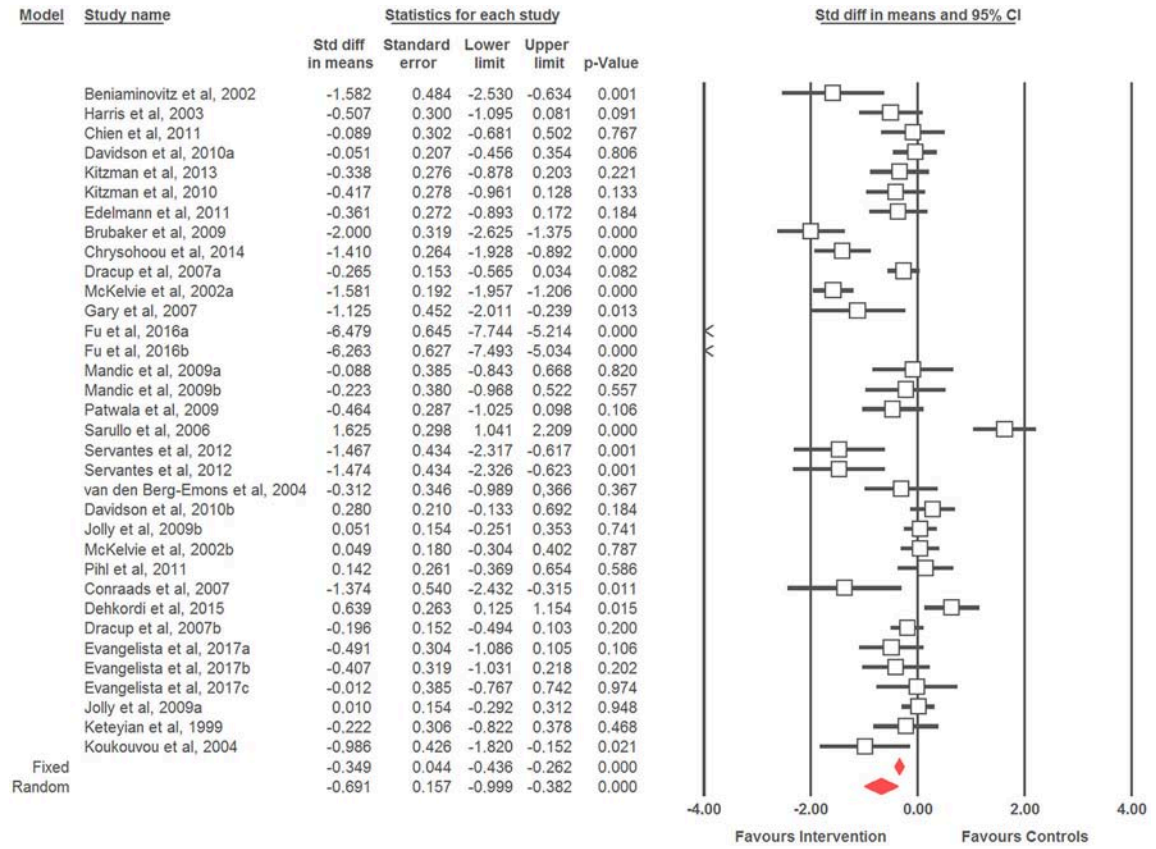

FIGURE 2 | Forest plot of physical training effect on quality of life of older patients with HF.

ES = 1.17; 95% CI = 0.45 to 1.89; p = 0.001) and combined aerobic and resistance training (small ES = 0.30; 95% CI = −0.49 to 1.10; $p = 0.450$), without significant difference between training modes ($Q = 2.49$; $p < 0.115$) (**Table 2**).

Dose-Response Relationship of Physical Training on Quality of Life, Aerobic Capacity, and Cardiac Function in Older Patients With Heart Failure

Findings From Meta-Regression Analysis

Table 3 shows the results of the meta-regression for the three training variables: duration of intervention, duration of single session, and weekly frequency. Only the duration of the intervention predicted QoL changes after physical training ($p = 0.006$) (**Figure 5**). The predictive influence of the remaining training variables was $p = 0.665$–0.996. Therefore, none of the examined training variables predicted aerobic capacity and cardiac function adaptation ($p = 0.280$–0.522) (**Table 3**).

Findings From the Univariate Analysis

Sub-analysis (**Table 4**) revealed that 12 weeks' training induced the greatest improvements in QoL (ES = −1.41; 95% CI = −2.20 to −0.61; $p = 0.001$). Regarding the frequency of training, 3–4 sessions per week induced the greatest improvements in QoL (ES = −0.98; 95% CI = −1.49 to −0.48; $p < 0.001$). Regarding single session duration, 31–45 min of training per session induced the greatest improvements in QoL (ES = −1.57; 95% CI = −2.53 to −0.60; $p = 0.001$).

DISCUSSION

The present meta-analysis summarizes evidence on the dose–response relationships between exercise (dose) and improvement of QoL, aerobic capacity, and cardiac function in (response) in older HF patients. The main findings of this meta-analysis were that (i) physical training exerted moderate effects on cardiac function, and small effects on QoL and aerobic capacity in older patients with HF and (ii) the training variable "duration" predicted the effects of physical training on QoL.

General Effectiveness of Physical Training

Previous meta-analyses have already examined the effect of physical training on QoL and aerobic performance and/or dose–response relationships for training variable (i.e., training intensity) in patients with HF (Pandey et al., 2015; Ostman et al., 2017). Pandey et al. (2015) showed that exercise training improves cardiorespiratory fitness (weighted mean difference = 2.72) and QoL (weighted mean difference = −3.97).

TABLE 2 | Effects of physical training on quality of life, aerobic capacity, and cardiac function in older patients with HF patients considering different moderating variables.

Independent variables	SMD	SE	95% CI	*p*	I^2 (%)	*df*	*Q value and (p) between groups*
QUALITY OF LIFE							
Gender							1.05 (0.592)
Males	−0.55	0.38	−1.29 to 0.19	0.148	52.92	1	
Females	−1.33	0.45	−2.01 to−0.24	**0.013**	0.0	0	
Combined	−0.69	0.17	−1.02 to −0.36	**<0.001**	92.12**	30	
Training mode							
aerobic	−1.04	0.32	−1.67 to−0.41	**0.001**	94.44**	17	4.20 (0.123)
Resistance	−0.17	0.32	−0.80 to 0.47	0.610	62.53	1	
Combined	−0.42	0.15	−0.71 to −0.13	**0.005**	82.48**	13	
AEROBIC CAPACITY							
Gender							
Females	0.04	0.42	−0.78 to 0.87	0.922	0.0	0	0.88 (0.349)
Combined	0.46	0.15	0.17 to 0.75	**0.002**	73.34**	9	
Training mode							
Aerobic	0.51	0.11	0.30 to 0.72	**<0.001**	0.00	5	15.54 (**<0.001**)
Resistance	1.71	0.34	1.03 to 2.39	**<0.001**	0.00	0	
Combined	0.15	0.20	−0.24 to 0.53	0.458	69.12*	3	
CARDIAC FUNCTION							
Training mode							
Aerobic	1.17	0.37	0.45 to 1.89	**0.001**	92.16**	9	2.49 (0.115)
Combined	0.31	0.41	−0.49 to 1.10	0.450	84.71**	2	

*SMD, standardized mean difference; SE, standard error; CI, confidence interval; p, significance value; I^2, heterogeneity index; df, degrees of freedom. Bold values indicate statistically significant values. *p < 0.05; **p < 0.01.*

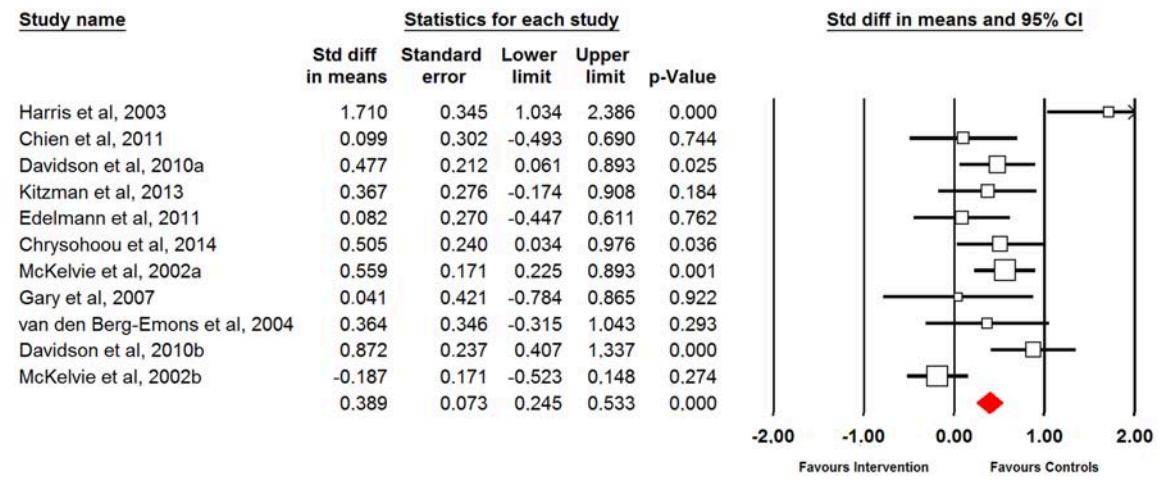

FIGURE 3 | Forest plot of physical training effect on aerobic capacity of older patients with HF.

These findings are in agreement with the results of Ostman et al. (2017) for QoL. However, as a novelty, our meta-analysis revealed that, aside from small improvements in QoL and aerobic capacity (i.e., 6-MWT), older patients with HF may achieve moderate improvements in cardiac function after physical training.

Type of Training/Intervention

Current findings indicate that aerobic training provide moderate improvements in QoL, compared to only small or trivial improvements after combined aerobic and resistance training and resistance training only, respectively. Ostman et al. (2017) reported that combined aerobic and resistance training or aerobic training alone improved QoL, whilst resistance training alone did not improve QoL. Similarly, Mandic et al. (2009) compared aerobic training vs. combined aerobic and resistance training in HF patients and reported that QoL was improved following aerobic training in compliant patients only. Cardiac rehabilitation programs focusing on

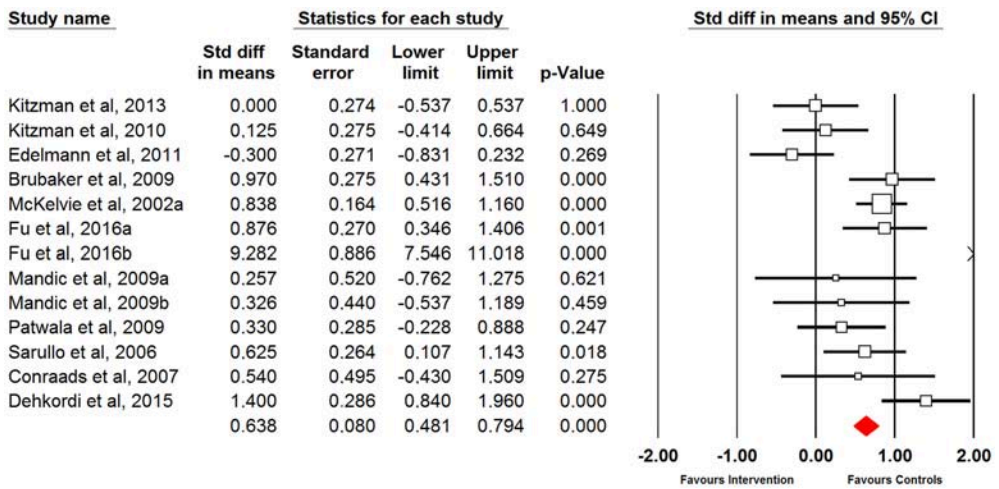

FIGURE 4 | Forest plot of physical training effect on cardiac function of heart failure patients.

TABLE 3 | Meta regression for training variables of different subscales to predict physical training effect on quality of life, aerobic capacity and cardiac function in in older patients with HF.

	Beta coefficient	Standard error	95% lower CI	95% upper CI	*Z*-value	*P*-value
QUALITY OF LIFE						
Duration of intervention	0.030	0.011	0.008	0.051	2.739	**0.006**
Duration of single session	0.000	0.012	−0.024	0.024	−0.004	0.996
Weekly frequency	0.068	0.158	−0.241	0.378	−0.433	0.665
AEROBIC CAPACITY						
Duration of intervention	−0.006	0.008	−0.022	0.010	−0.699	0.484
Duration of single session	0.006	0.010	−0.013	0.026	0.641	0.522
Weekly frequency	0.161	0.149	−0.131	0.452	1.079	0.280
CARDIAC FUNCTION						
Duration of intervention	−0.053	0.079	−0.209	0.102	−0.675	0.500
Duration of single session	−0.069	0.083	−0.232	0.094	−0.825	0.409
Weekly frequency	1.579	1.607	−1.570	4.727	0.983	0.326

Bold values indicate statistically significant values.

aerobic training have resulted in reduced symptoms (i.e., pain, lower extremity edema, coughing, and breathe problems) and enhance functional capacity (Pollentier et al., 2010) following implementation.

Concerning changes of aerobic capacity (i.e., 6-MWT), the present meta-analysis revealed that resistance training produced greater performance improvements than aerobic and combined training. This may be explained by the beneficial effects of resistance training on running economy and strength, which results in greater recruitment of type I fibers for the same submaximal load, better muscular coordination, and therefore better mechanical efficiency, whilst likely concomitantly enhancing aerobic capacity (Hartman et al., 2007; Yamamoto et al., 2008, 2010; Cadore et al., 2011). Contrastingly, Wood et al. (2001) reported greater improvements in repeated chair stand after combined training, vs. with resistance-only and endurance-only training in healthy older individuals. Furthermore, another review revealed that resistance and aerobic training have a similar effect on aerobic capacity in older adults (Liu and Latham, 2009). As such, practitioners/physicians should implement resistance training to improve 6-MWT performance, and thus functional capacity in patients with HF.

This meta-analysis showed that aerobic training had the largest effect on cardiac function and mechanisms may be related to presence of mediators such as nitric oxide, which increase cardiac vagal tone after aerobic exercise, and angiotensin II, which inhibits cardiac vagal activity, and the adaptation of the autonomic nervous system in favor of parasympathetic dominance (Kingwell, 2002; Billman and Kukielka, 2007). Previous meta-analysis has suggested that aerobic or combined exercise, but not resistance exercise, may be effective in improving cardiac function in HF patients (Haykowsky et al., 2007). Delagardelle et al. (2002) found a greater improvement of aerobic performance and systolic function after four months of combined aerobic and resistance training than aerobic training in HF patients. Of note, in the present meta-analysis, the absence of significant difference in cardiac function between aerobic and

combined training may be due to the high heterogeneity among included studies.

Effects and Dose–Response Relationships Following Physical Training

Training Variables (Training Duration in Weeks, Weekly Training Frequency, Session Duration)

The current meta-analysis substantially advances the literature compared to previous reviews (Piepoli et al., 2004; Smart and Marwick, 2004; Giuliano et al., 2017; Ostman et al., 2017), as we provide the dose–response relationships of physical training variables such as frequency, duration, and volume with training adaptations. Included studies showed large variation in training variables whereby training periods ranged from 4-weeks to 1-year, frequencies from one to five times/week, and duration of training sessions from 10 to 65 min. The characterization of dose–response relationships revealed that, when considered individually, and not as complete training protocol, training periods of 12 weeks, a frequency of 3–4 sessions per week, and durations of 31–45 min of a single training session, were the most effective training variable specifics for improvements in QoL.

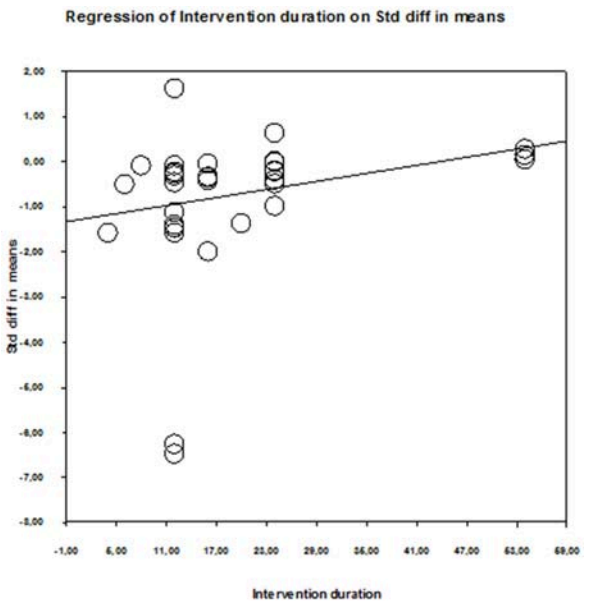

FIGURE 5 | Scatter plot of regression analysis showing influence of intervention duration on the quality of life of older patients with HF.

Dose-response analyses revealed that shorter training durations are more effective at improving QoL. Specifically, sub-group analysis showed that physical training lasting 12 weeks is most effective for improving global QoL in older HF patients. As illustrated in **Table 3**, longer training periods produced lower ES, compared to shorter training (i.e., 4–16 weeks) periods. Davidson et al. (2010) reported significant differences between intervention and control groups in the measure of QoL (MLWHFQ) at 3 months, whilst there were no differences between groups at 12 months. Health Canada (1999), American College of Sports Medicine (ACSM) and the American Heart Association (AHA) (Nelson et al., 2007) recommended 30–60 min a day of aerobic activity of moderate-intensity for several months to promote and maintain health, and reduce the risk of chronic disease, premature mortality, functional limitations, and disability. Shorter periods of training are usually accompanied by greater adherence rates (Conraads et al., 2012; De Maeyer et al., 2013). In fact, only minor group could reach the long-term period of >6-month and could reach the 120 min per week (De Maeyer et al., 2013). Despite that, other authors relate improvements of maximal oxygen uptake to the period of training (Tabet et al., 2008) and absence of changes in this parameter is strongly correlated with cardiac risk. With regard to period of training, Lloyd-Williams et al. (2002) found that short-term physical activity is beneficial to patients with chronic heart failure and most patients experience improvement in their QoL.

TABLE 4 | Effects of physical training on quality of life considering different moderating variables.

Independent variables	SMD	SE	95% CI	*p*	I^2(%)	*df*	*Q value and (p) between groups*
DURATION OF INTERVENTION							
4–8 weeks	−0.64	0.38	−1.38 to 0.10	0.088	70.83*	2	20.59 (**p < 0.001**)
12 weeks	−1.41	0.41	−2.20 to−0.61	**0.001**	95.53**	12	
16 weeks	−0.61	0.31	−1.22 to 0.00	**0.050**	85.47**	4	
5–6 months	−0.23	0.15	−0.52 to 0.07	0.132	62.40*	8	
1 year	0.11	0.10	−0.08 to 0.30	0.251	0.00	3	
WEEKLY FREQUENCY							
1	0.11	0.17	−0.21 to 0.44	0.498	20.40	1	13.89 (**0.003**)
2–3	−0.56	0.43	−1.40 to 0.28	0.194	92.62**	3	
3–4	−0.98	0.26	−1.49 to−0.48	**<0.001**	93.54**	20	
5	−0.13	0.10	−0.33 to 0.08	0.224	15.97	5	
DURATION OF SINGLE SESSION							
20–30 min	−0.31	0.19	−0.68 to 0.06	0.105	87.48**	14	6.19 (**0.045**)
31–45 min	−1.57	0.49	−2.53 to−0.60	**0.001**	96.72	7	
46–65 min	−0.68	0.25	−1.18 to−0.18	**0.007**	83.90**	8	

*SMD, standardized mean difference; SE, standard error; CI, confidence interval; p, significance value; I^2, heterogeneity index; df, degrees of freedom. Bold values indicate statistically significant values. *p < 0.05; **p < 0.01.*

Our meta-analysis has several limitations that warrant discussion. Firstly, we computed meta-regression and univariate analyses to identify effective dose-response relationships. Of note, findings from univariate analyses must be interpreted with caution because training variables were computed as single factors irrespective of potential between-variable interactions (Gebel et al., 2018). Secondly, we have not performed univariate analyses for aerobic capacity and cardiac function due to the small number of studies. Thirdly, due to limited number of studies examining the effects of physical training in female and male patients, we were not able to compare sexes.

CONCLUSIONS

Physical activity is an effective therapeutic method in older patients with HF, with small to moderate effects on QoL, aerobic capacity, and cardiac function, irrespective of sex and training mode. Dose–response analyses showed that none of the training variables predicted changes in aerobic capacity or cardiac function. However, for QoL, the meta-regression indicated that the training duration significantly predicted the observed improvement, with shorter training duration showing larger improvements.

AUTHOR CONTRIBUTIONS

MS, RR-C, AP, LH, NB, and MS study concept and design. MS, RR-C, AP, LH, NB, and MS analysis and interpretation of data. MS, RR-C, AP, LH, NB, and MS final approval of the version to be published. MS, RR-C, AP, LH, NB, and MS agreement to be accountable for all aspects of the work.

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CASE REPORT
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Pacing During and Physiological Response After a 12-Hour Ultra-Marathon in a 95-Year-Old Male Runner

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In recent years, outstanding performances of elderly people up to 100 years have been reported. In this case study, pacing during and recovery after a 12-h ultra-marathon were described for a 95-year old runner. The athlete achieved a total distance of 52.987 km. Pacing followed a parabolic pattern (U-shaped), where the speed decreased till the middle of the race and then increased. However, no end spurt was observed. A large main effect of lap quartile on speed was observed, where the second quartile was slower than the first quartile and forth. The smallest variability was shown in the first quartile and the largest in the second quartile. During recovery, erythrocytes, hemoglobin and hematocrit increased whereas thrombocytes and leucocytes decreased. CRP, GOT, GPT, y-GT, CK, and LDH were increased post-race and decreased to reference range during recovery. Also, creatinine and urea decreased during recovery. Creatinine clearance increased during recovery. Sodium increased during recovery and remained constantly within the reference range. During recovery body fat and visceral fat mass decreased, whereas body water and lean body mass increased. In summary, a 95-year-old man was able to run during 12 h using a U-shaped pacing and achieving a total distance of nearly 53 km. Increased selected hematological and biochemical parameters returned to pre-race values within a recovery phase of 5 days.

Keywords: master athlete, elderly, endurance, performance, running

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INTRODUCTION

Ultra-marathon running is of high popularity where recent findings showed an increase in both the number of races (Cejka et al., 2014) and successful finishers (Knechtle et al., 2018b). It is well documented that the increase in the number of successful ultra-marathoners in recent years is due to the increase in both women (Hoffman, 2010) and age group athletes (Knechtle et al., 2014).

While it is well-known that age group marathoners can compete late in their life until the age of 70 years (Jokl et al., 2004; Lepers and Cattagni, 2012), 80 years (Brendle et al., 2003) or even to 90 years (Addison et al., 2015; Ahmadyar et al., 2016), very little is known regarding elderly ultra-marathoners of very high ages (Knechtle and Nikolaidis, 2018a). We know from large data set

analyses that elderly runners of 70 years (Rüst et al., 2015) up to 85 years (Rust et al., 2014) are able to finish an ultra-marathon.

Pacing is an important aspect for a successful race finish in marathon (Nikolaidis and Knechtle, 2017a,b, 2018b,c; Diaz et al., 2018) and ultra-marathon (Lambert et al., 2004) running. We recently got new insights in the pacing of master marathoners (Nikolaidis and Knechtle, 2017a,b, 2018b), but very little is known for the pacing of master ultra-marathoners (Knechtle et al., 2015; Rüst et al., 2015; Knechtle and Nikolaidis, 2018a; Knechtle et al., 2018a). In a recent case report pacing and recovery phase of a 94-year-old runner in a 6-h ultra-marathon has been reported (Knechtle and Nikolaidis, 2018a), but no longer ultra-marathon duration has been investigated for an ultra-marathoner of this age. In the present case report we investigate the same runner one year later at the age of 95 years in a 12-hrun.

It is well-known that ultra-marathon running leads to considerable acute changes in biomarkers deviating from reference values in specific organs or organ systems such as skeletal muscles, heart, liver, kidney, immune, and endocrine system (Knechtle and Nikolaidis, 2018b). Usually, these changes are temporary and depend on intensity and duration of the performance, and generally, they normalize within a few days after the race (Knechtle and Nikolaidis, 2018b); thus, they should be considered as acute physiological responses to ultra-endurance exercise rather than pathological.

In this case study we investigated, first, the pacing during a 12-h ultra-marathon in a 95-year-old runner and, second, the recovery phase after the race. Regarding the age group world records in ultra-marathon running[1] and existing scientific reports, no person at this age ever competed in such a race. Moreover, although the age-related differences in ultra-marathon running performance have been well-documented (Knechtle et al., 2014; Nikolaidis and Knechtle, 2018d), no study has been ever conducted in a 95-year-old runner with regards to the variation of blood physiology during an ultra-marathon race and recovery.

MATERIALS AND METHODS

Ethics Statement

This case report was approved by the ethical review board of Kanton St. Gallen, Switzerland. The runner provided his written informed consent to the analysis and publication of his data.

The Runner and the Race

Our runner is born in 1923 and started running after his retirement at the age of 65 years. He mainly competed in short distance running races like 5 km, 10 km, and switched later to half-marathon (Knechtle et al., 2010). At the age of 90 years, he successfully completed his first marathon (Mueller et al., 2014). He is the current record holder of the European record in half-marathon and marathon running in age group in M90[2]. In the preparation of this race, he trained 5 days per week on weekdays with a break during the weekend. The daily training varied from 5 to 10 km. One of the training units was together with a group of recreational runners.

Regarding his old age (95 years old), a mild to moderate arterial hypertension is treated with an ACE-inhibitor (angiotensin converting enzyme inhibitor) (5 mg Lisitril®) and osteopenia is treated with daily intake of 1000 mg calcium (CALCIUM Sandoz® Brausetabl 1000 mg).

On May 12, 2018, the athlete started in the 12-h run at midnight within the 'Sri Chinmoy 12 + 24 Stunden-Lauf' held annually in Basel, Switzerland (Stunden-Lauf, 1988). The course is a flat circuit (1101.4 m) on asphalt, lit at night and officially measured by an IAAF-measurer Grade B. The race has the IAU bronze label since 2007. Lap control consists of an electronic timekeeping (two chips at the back of the race number) plus a personal lap counter at the counting stating and a video camera as backup. After passing each lap the runners have a well visible digital clock. With an electronic chip attached to the race number, the time each lap is measured by an official timekeeping company. When the athlete enters the last lap, he takes a little flag with the starting number on it and leaves it at the final whistle on the edge of the road. The organizer then measures the distance so that the full distance can be measured exactly.

The organizer offers a buffet at the runners each round pastas drinks (e.g., water, tea warm, caffeinated drinks, isotonic sports drink, broth, malt beer, red bull, and coffee) as well as solid foods (e.g., pasta, potatoes, bread with various pads such as cheese or jam, salt brezels, chips, peanuts, bars, cakes, chocolate, biscuits, fruits such as bananas, oranges, watermelons, and grapes). The runners can also arrange a food-stuffing along the route themselves and feed themselves or by accompanying persons. The runner was supported by a personal female support providing him in the beginning of the race in the morning hot coffee and bread with jam. Later during the race he drank Coca Cola® and ate some fruits. The runners can also take individual breaks. The runner made one short break in about the middle of the race.

Measurements

Before the competition, we measured body mass, percentage of body fat, fat-free mass and percentage of body water using a bioelectrical impedance scale Tanita BC-545 (Tanita, Arlington Heights, IL, United States) to repeat the measurements after the run and for the 5 days after the race. The reliability and validity of this device has recently been shown (Wang and Hui, 2015).

At the same time points, capillary blood samples at a fingertip were drawn. We measured hemoglobin, hematocrit, leukocytes, platelets, C-reactive protein, creatin-kinase, LDH (Lactate dehydrogenase), GPT (glutamate pyruvate transaminase), GOT (aspartate aminotransferase), γ-GT (gamma-glutamyltransferase), creatinine, potassium and sodium. Hematological analysis [erythrocytes, hemoglobin, hematocrit, thrombocytes, mean cell volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC) and leucocytes] was performed using ABX Micros CRP 200 medical lab (HORIBA Medical, Montpellier, France) and

[1] www.iau-ultramarathon.org/

[2] http://european-masters-athletics.org/files/EV_180630.L%C3%85.pdf

the analysis of the serum parameters using Fuji Dri-Chem 4000i analysis system (FUJIFILM Corporation, Tokyo, Japan). Both laboratory machines undergo regular internal[3] and external[4] quality controls. Creatinine clearance was estimated using the Cockcroft and Gault formula (Cockcroft and Gault, 1976).

Data Analysis

Both graphical and numerical approaches were used to examine the normality of the data, i.e., visual inspection of normal Q-Q plots and the Shapiro-Wilk test, respectively. Accordingly, parametric statistics were applied. The variation of speed by lap was examined using a fourth degree polynomial regression analysis and the relationship between these variables was estimated by coefficient of determination (R^2). Laps were grouped into quartiles, i.e., 1–12, 13–24, 25–36, and 37–48 laps. A repeated measures analysis of variance examined differences in speed among quartiles. 95% confidence intervals (CI) were calculated for mean differences among quartiles. The magnitude of differences was estimated using eta square classified as small ($0.010 < \eta^2 \leq 0.059$), medium ($0.059 < \eta^2 \leq 0.138$), and large ($\eta^2 > 0.138$) was used. The relationship among variables was examined using Pearson correlation coefficient r, whose magnitude was evaluated as trivial ($r < 0.10$), small ($0.10 \leq r < 0.30$), moderate ($0.30 \leq r < 0.50$), large ($0.50 \leq r < 0.70$), very large ($0.70 \leq r < 0.90$) and perfect ($r \geq 0.90$). The acceptable type I error was set at $p < 0.05$.

RESULTS

Performance

The athlete achieved a total distance of 52.987 km. As shown in **Figure 1**, the pacing followed a parabolic pattern (U-shaped), where the speed decreased till the middle of the race and then increased. However, no end spurt was observed. A large main effect of lap quartile on speed was observed ($F_{2,077} = 8.193$, $p = 0.002$, $\eta^2 = 0.427$), where the second quartile was slower than the first quartile (−0.88km/h; 95% CI −1.51, −0.25) and forth quartile (−0.93km/h; 95% CI −1.85, 0) (**Figure 2**). The smallest variability was shown in the first quartile and the largest in the second quartile.

Laboratory Values Before and After the Race

The indices of blood physiology during the race and recovery were presented in **Figure 3**. Hematocrit and MCHC were out of the normal range. During recovery, erythrocytes, hemoglobin and hematocrit increased whereas thrombocytes and leucocytes decreased. The indices of biochemistry were presented in **Figure 4**. CRP, GOT, GPT, y-GT, CK, and LDH were increased post-race. During recovery, these variables decreased to normal range where also creatinine and urea decreased. Creatinine clearance increased during recovery. Sodium increased during recovery and remained always within the reference range.

[3]www.qualab.swiss/Interne-Qualitaetskontrolle.htm

[4]www.qualab.swiss/Externe-Qualitaetskontrolle.htm

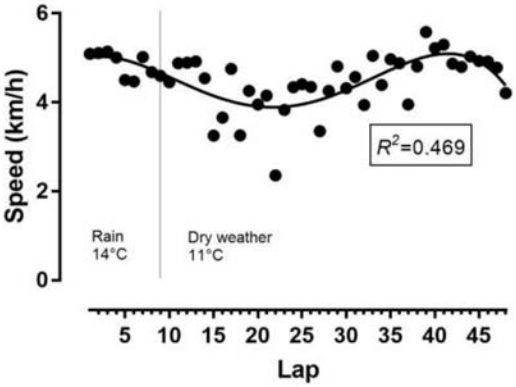

FIGURE 1 | Variation of speed by lap. Temperatures referred to weather conditions during the race. R^2, coefficient of determination.

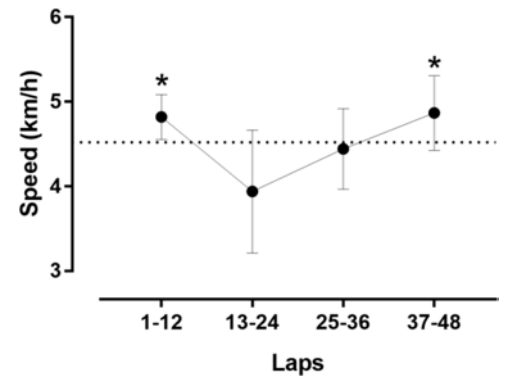

FIGURE 2 | Speed by quartiles of laps.*different from the second quartile at $p < 0.05$. Error bars represent standard deviations.

Changes in body composition during race and recovery were presented in **Figure 5**. Post-race, body fat, and visceral fat mass decreased, whereas body water and lean body mass increased.

Correlations

The change in CRP correlated almost perfectly with the change in CK ($r = 0.930$, $p = 0.002$) and very largely with the change in LDH ($r = 0.770$, $p = 0.043$), whereas the change in Lc correlated almost perfectly with the change in CK ($r = 0.901$, $p = 0.006$), but not with the change in LDH ($r = 0.632$, $p = 0.128$).

The change in body water was not related to the change in hemoglobin ($r = -0.433$, $p = 0.332$) or the change in hematocrit ($r = -0.436$, $p = 0.328$). The change in body water correlated very largely with the change in sodium ($r = 0.848$, $p = 0.016$), but not with the change in creatinine ($r = -0.421$, $p = 0.347$), creatinine clearance ($r = 0.304$, $p = 0.507$), urea ($r = -0.267$, $p = 0.563$), potassium ($r = -0.565$, $p = 0.186$).

The changes in CK and LDH as variables of skeletal muscle damage were not related to the change in creatinine ($r = 0.615$, $p = 0.142$ and $r = 0.297$, $p = 0.517$, respectively) or the change

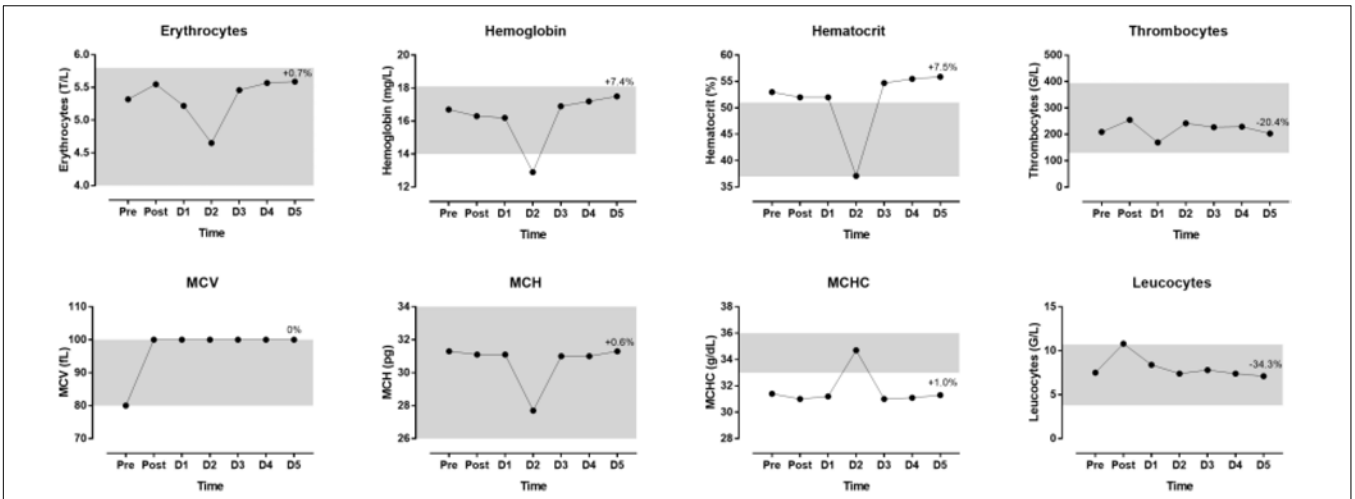

FIGURE 3 | Blood physiology indices during the race and recovery. The shadowed area represents normal reference values. The percentage value over D5 point denotes change from post-race value. D, day post-race.

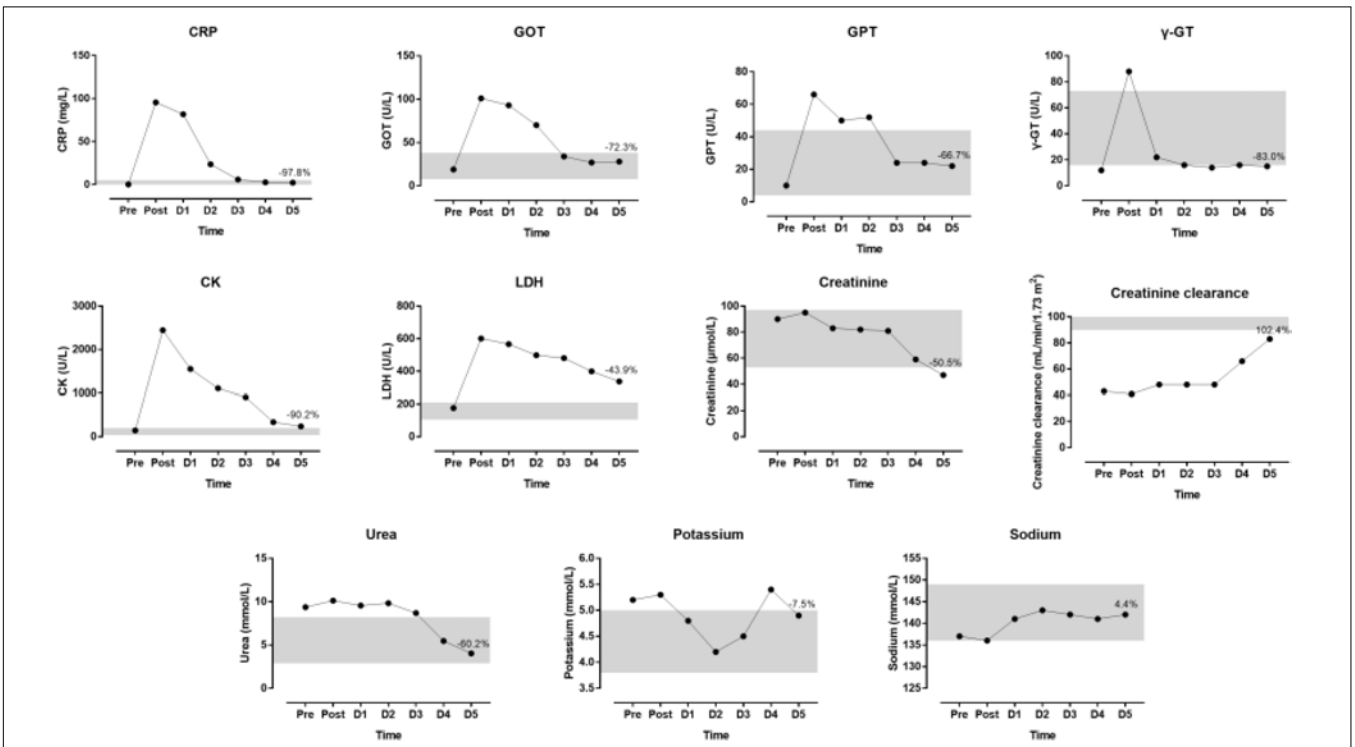

FIGURE 4 | Biochemical indices during the race and recovery. The shadowed area represents normal reference values. The percentage value over D5 point denotes change from post-race value. D, day post-race.

in creatinine clearance ($r = -0.565$, $p = 0.186$ and $r = -0.302$, $p = 0.510$).

DISCUSSION

In this case report, it was found that (i) a 95-year-old man was able to run during 12 h using a U-shaped pacing and achieving a total distance of nearly 53 km and (ii) increased selected hematological and biochemical parameters returned to pre-race values within a post-race recovery phase of 5 days.

The present ultra-marathoner showed a U-shaped pacing where running speed decreased in the first 6 h to increase in the second 6 h. Furthermore, the second quartile of the run was slower than the first and fourth quartile. Generally,

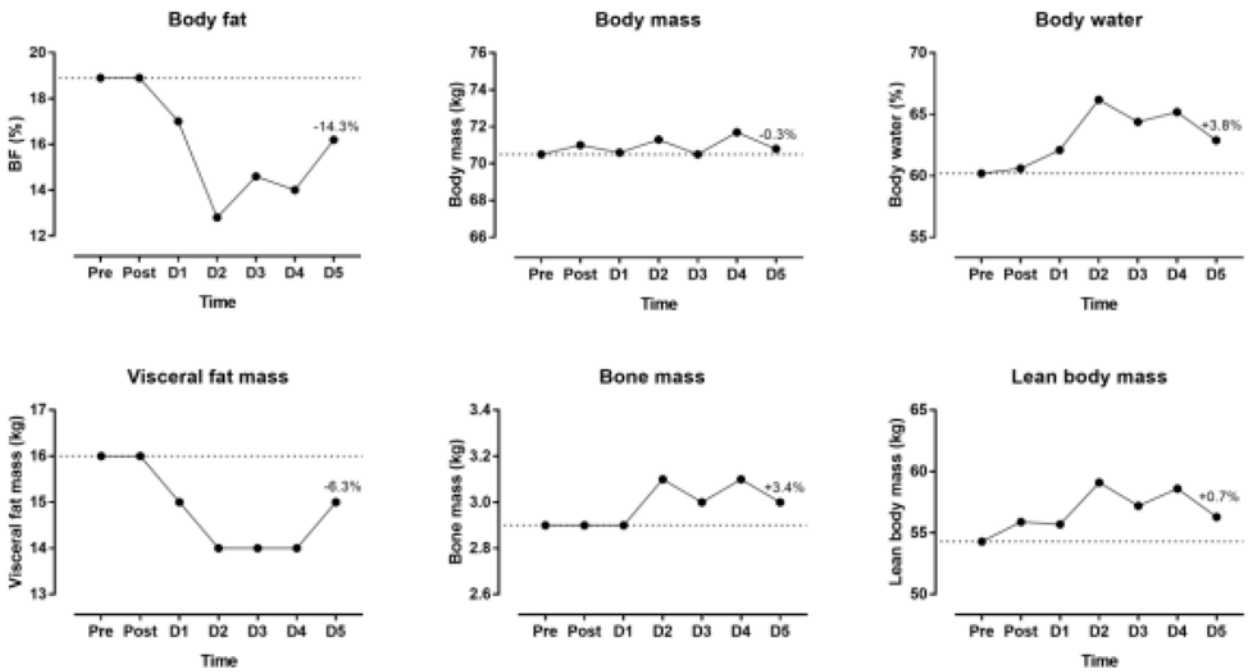

FIGURE 5 | Body composition during the race and recovery. The dashed line represents the pre-race values. The percentage value over D5 point denotes change from post-race value. D, day post-race.

ultra-marathoners show a positive pacing (i.e., slowing down) during the race (Lambert et al., 2004; Hoffman, 2014; Bossi et al., 2017). We might assume that the present ultra-marathoner was aware of his performance in 6-h ultra-marathon running (Knechtle and Nikolaidis, 2018a) and preserved energy for the second half of the race.

The observed physiological responses to the ultra-endurance race were in agreement with the existing literature indicating that regardless of the age of ultra-endurance runners, negative responses of the function of internal organs and skeletal muscles to exercise occurred during the race similarly for younger and older runners (Jastrzebski et al., 2015c). In addition, these responses depended on the length of the distance covered and were greatest at the end of the ultra-endurance exercise.

We found that increased hematological and biochemical parameters returned nearly all to baseline values within the recovery phase of 5 days. It is well-known that biomarkers of skeletal muscle, liver, and kidney damage increase partially dramatically during an ultra-marathon (Jastrzebski et al., 2015a; Shin et al., 2016) where higher age and faster running speeds lead to more pronounced damage in both liver and kidney. However, like in the present case report, all increased values of biomarkers return within a few days to base line values (Kłapcińska et al., 2013; Knechtle and Nikolaidis, 2018b).

An interesting finding was that erythrocytes, hemoglobin, hematocrit and MCH were very low on day 2 where MCHC was increased that day. These changes might be typical for an ultra-marathon and revealed increased rates of destruction of erythrocytes, which was in agreement with the concept of sports anemia. It has been suggested that sports anemia in endurance athletes might be due to hemolysis owing to mechanical trauma and oxidative injuries of erythrocytes (Wu et al., 2004). For instance, this hemolysis might be caused by mechanical trauma when erythrocytes passed through capillaries in contracting muscles and by compression of erythrocytes (e.g., in foot soles during running) (Mairbaurl, 2013). In a 24-h ultra-marathon, significant declines in erythrocytes, hemoglobin and hematocrit were detected 2 days and 9 days after the race (Wu et al., 2004). Leucocytes were increased post-race and returned then to pre-race values. Although the changes in leucocytes during ultra-marathon running might be due to an immune response (Zakovska et al., 2017), the increase in leucocytes after the race is most probably due to an inflammatory process (Jee and Jin, 2012).

Interestingly, total body water was highest that day. Most probably the athlete was much diluted that day. Furthermore, we found an increase in total body water after the race where the increase was not related to changes in biomarkers of renal function but to the increase in sodium. Fluid conservation after an ultra-marathon is well-known (Fellmann et al., 1989) and most likely due to endocrine regulation of plasma sodium concentration (Brge et al., 2011). Indeed, sodium concentration increased during recovery and remained always within the reference range.

During recovery, we found correlations between biomarkers of inflammation (e.g., CRP, Lc) with biomarkers of skeletal muscle damage (e.g., CK, LDH). It should be highlighted that inflammation was indicated by the increase of CRP and leukocytes across race suggesting interplay between hematological (leukocytes) and biochemical (CRP) parameters

(Kim et al., 2009). Moreover, it was well-known that biomarkers of both skeletal muscle damage and inflammation increase during an ultra-marathon (Kim et al., 2007). During recovery after an ultra-endurance performance such as an Ironman triathlon, biomarkers of inflammation persisted for 5 days after the race, most likely reflecting incomplete muscle recovery (Neubauer et al., 2008).

The changes in biomarkers of skeletal muscle damage (e.g., CK, LDH) were not related to changes of renal function (e.g., creatinine, creatinine clearance) during recovery. An ultra-marathon often leads to a temporary reduction in renal function (Knechtle and Nikolaidis, 2018b). The prevalence of an acute kidney injury in ultra-marathon running can reach 50% of all runners (Lipman et al., 2017). It is assumed that skeletal muscle damage leads to an increase in muscle proteins (e.g., myoglobin). This increase leads to rhabdomyolysis (Schiff et al., 1978) and, consequently, to renal failure (Uberoi et al., 1991).

One might assume that the increase in selected hematological and biochemical variables might be higher in an older athlete compared to a younger one. The change in laboratory variables seems more dependent upon the length of the performance and the intensity than the age of the runner (Del Coso et al., 2013; Shin et al., 2016). Regarding half-marathon and marathon running, marathon running lead to more pronounced responses in myoglobin, CK-MB-mass, ALT, AST, lactate and phosphate (Niemela et al., 2016). Regarding age, elevations in troponin levels are observed only in young participants (<30 years), most strikingly in those younger than 20 years of age (Niemela et al., 2016). Overall, the responses in the increases in these selected hematological and biochemical variables are in line with other reports (Knechtle and Nikolaidis, 2018b).

The findings of the present case study were of great theoretical and practical value, especially considering the increased participation in ultra-marathon races during the last years (Nikolaidis and Knechtle, 2018a,d). The ability of a 95-year-old man to finish a 12-h-run might be first of all to his adaptive abilities acquired during many years of training. Moreover, the long-term adaptations to ultra-endurance exercise might include the exceptional ability to regulate the intensity of his effort in order not to allow to be interrupted. These relationships were particularly evident from changes in the acid-base balance and lactate concentration in the blood during exercise. Ultra-endurance runners do not allow their body to undergo deep changes, e.g., in saturation or blood acidification by temporarily slowing down the pace of the race. On the other hand, running above their limits would probably interrupt the effort after several minutes as a result of muscle acidification (Jastrzebski et al., 2015b). With regard to the effect of recovery, it is clearly observed that in a healthy person the mechanisms of recovery of organs' physiological function are efficient regardless of age, which is confirmed by all authors.

Although this was the first study to show that a 95-year-old man was able to run during 12 h, master athletes of very old ages (i.e., 90 years and 100 years) were extending their limits (Lepers and Stapley, 2016) and were able to achieve outstanding performances (Lepers et al., 2016). Future studies would be needed to expand our knowledge about athletic performances of elderly people up to 100 years using larger sample sizes. However, a limitation for full interpretation of the laboratory analyses was the lack of detailed analysis of fluid and food intake during both the race and the recovery period. On the other hand, strength of this study was that it examined the post-race recovery for five consecutive days providing detailed information about the daily variation of blood physiology, whereas most existing research focused on changes during an ultra-marathon race but not on recovery (Zakovska et al., 2017). Furthermore, recovery was previously studied using few post-race assessment days, e.g., the second and ninth post-race day in a 24-h ultra-marathon (Wu et al., 2004) in contrast to the daily assessment of blood physiology for five consecutive post-race days in the present study.

CONCLUSION

In conclusion, a 95-year-old man was able to run – despite the fatigue – during 12 h using a U-shaped pacing and achieving a total distance of nearly 53 km. Increased selected hematological and biochemical parameters returned to pre-race values within a post-race recovery phase of 5 days. It seemed that a person at this old age recovered within 5 days from an 12-h ultra-marathon.

AUTHOR CONTRIBUTIONS

BK and PN conceived and designed the study. BK collected data. BK, ZJ, TR, and PN analyzed and interpreted the data and drafted the manuscript. BK, ZJ, TR, and PN revised the manuscript and approved the final version.

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REVIEW
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Mitochondria as a Target for Mitigating Sarcopenia

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Sarcopenia is the loss of muscle mass, strength, and physical function that is characteristic of aging. The progression of sarcopenia is gradual but may be accelerated by periods of muscle loss during physical inactivity secondary to illness or injury. The loss of mobility and independence and increased comorbidities associated with sarcopenia represent a major healthcare challenge for older adults. Mitochondrial dysfunction and impaired proteostatic mechanisms are important contributors to the complex etiology of sarcopenia. As such, interventions that target improving mitochondrial function and proteostatic maintenance could mitigate or treat sarcopenia. Exercise is currently the only effective option to treat sarcopenia and does so, in part, by improving mitochondrial energetics and protein turnover. Exercise interventions also serve as a discovery tool to identify molecular targets for development of alternative therapies to treat sarcopenia. In summary, we review the evidence linking mitochondria and proteostatic maintenance to sarcopenia and discuss the therapeutic potential of interventions addressing these two factors to mitigate sarcopenia.

Keywords: skeletal muscle, aging, sarcopenia, exercise, mitochondria, treatment

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INTRODUCTION

The Centers for Disease Control (CDC) projects that the proportion of adults older than 65 years of age in the US population will grow from 12.4 to 19.6% reflecting the aging baby boom generation and declining birth rates in the United States. Furthermore, the population of persons older than 80 years is expected to more than double from 9.3 million in 2000 to 19.5 million in 2030 (Centers for Disease Control and Prevention (CDC), 2003). This aging phenomenon is not unique to the United States since the number of individuals aged 65 years and older is expected to nearly double from 6.9% of the world population in 2000 to 12.0% by 2030 (Beard et al., 2016). Revisions to such predictions even suggest that by 2050, the population of those over the age 65 in the United States will be up to 108 million, 25.8% of the predicted population (Olshansky et al., 2009).

Since the aged population is increasing globally, the prevalence of sarcopenia, the age-related loss of skeletal muscle mass and function, is likely to increase as well. Global prevalence of sarcopenia is difficult to measure in part due to changing consensus of what constitutes the diagnosis of sarcopenia. In 1998, Baumgartner et al. defined sarcopenia as the age-associated loss of skeletal muscle mass two standard deviations below a healthy population (Baumgartner et al., 1998). Based strictly on skeletal muscle mass loss, Baumgartner and colleagues estimated

that 24% of individuals less than 70 years of age have sarcopenia while 50% of those over 80 years of age had sarcopenia (Baumgartner et al., 1998). More recent analyses find widely discrepant disease incidence with NHANES data collected between 1999 and 2004 reporting that 27.8 and 19.3% of men and women at least 60 years of age were sarcopenic (Janssen et al., 2002). Other estimates are as high as 35.4 and 52.5% for women over 60 and 80 years of age, respectively, and 75.5 and 88.1% for men over 60 and 80 years of age (Batsis et al., 2014). Surprisingly, there is a paucity of data that detail the economic burden of sarcopenia, although an analysis over a decade old found that the healthcare cost of sarcopenia in the United States was an estimated $18.5 billion per year (Janssen et al., 2004).

Skeletal muscle is the largest organ in the human body and plays a key role in posture and capacity for locomotion, as well as serving as a bona fide endocrine organ (Pedersen and Febbraio, 2012). As such, skeletal muscle dysfunction has detrimental effects on many aspects of human health for older adults. Epidemiological studies have found that sarcopenia increases the overall risk for mortality (Landi et al., 2013;

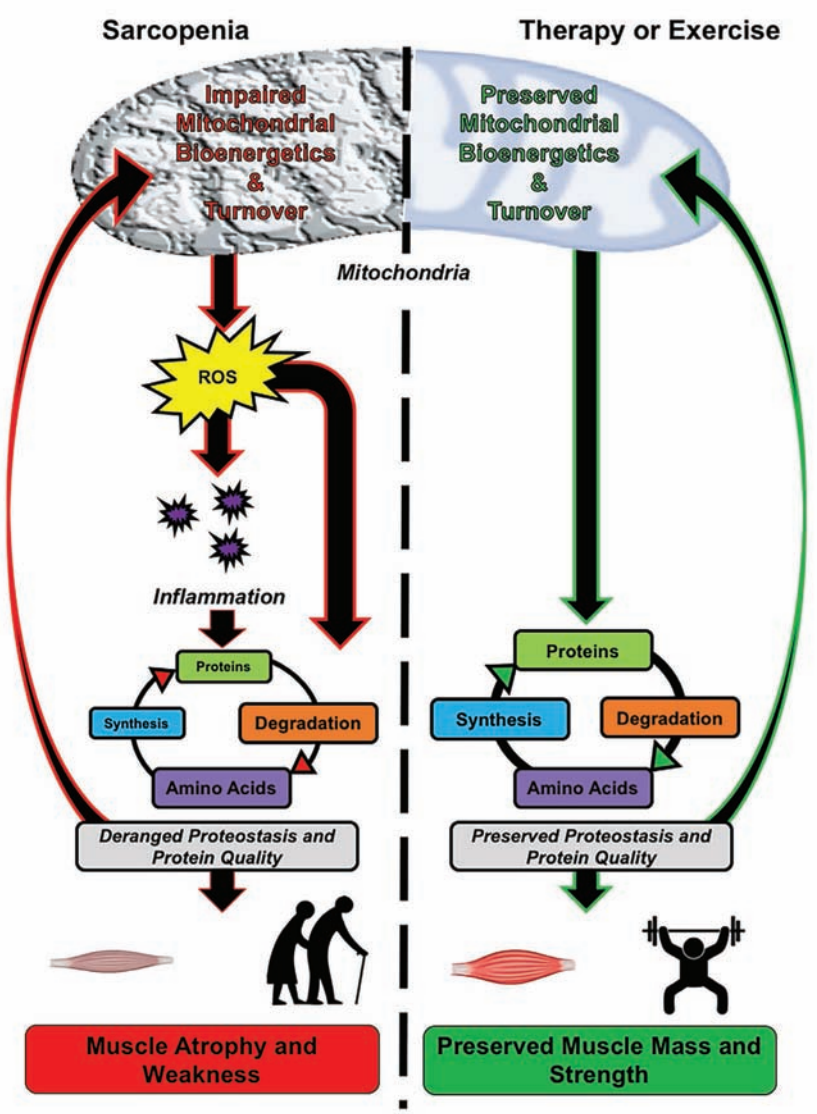

FIGURE 1 | Role of mitochondrial bioenergetics and proteostasis in mediating skeletal muscle quality in older adults. *Left panel,* Sarcopenia is associated with mitochondrial dysfunction, which encompasses impaired bioenergetics and turnover. The impairment results in increased reactive oxygen species (ROS) generation and chronic low-grade inflammation, leading to impaired muscle proteostasis. The derangement in proteostasis impedes mitochondrial turnover, resulting in an accumulation of dysfunctional mitochondria and further exacerbation of organelle and tissue dysfunction. *Right panel,* Targeting mitochondrial bioenergetics and turnover by therapeutics and exercise impedes the age-associated rise in ROS and systemic inflammation, which results in the maintenance of muscle proteostasis. The maintained protein turnover allows for the removal of damaged proteins, such as dysfunctional mitochondria and damaged contractile proteins, while also synthesizing new functional proteins. Collectively, this leads to preservation of mitochondrial quality, muscle mass, and strength.

Batsis et al., 2014). In part, this is because sarcopenia increases the risk of developing mobility disabilities, leading to impairment in activities of daily living by twofold (Janssen et al., 2002), and risk of falls by three times (Landi et al., 2013). The loss of muscle mass and function is not exclusive to postural/locomotor muscle groups, as myopathy of key inspiratory muscles also occurs with aging, resulting in respiratory failure (Kelley and Ferreira, 2017). The increased risk of disability from respiratory failure has led to greater hospitalization of older adults (Verissimo et al., 2015; Kelley and Ferreira, 2017). The combined effect of sarcopenia and hospitalization further exacerbates muscle dysfunction, as older adults do not adequately recover from bed rest (Suetta et al., 2009, 2013; Hvid et al., 2010), which contributes to reduced functionality and ambulation upon discharge, and leads to loss of independence, nursing home placement, and increased risks of falls (Fortinsky et al., 1999; Mithal et al., 2013). In addition to physical disability, sarcopenia contributes to the development of cardiovascular and metabolic diseases because of its involvement in substrate metabolism (Batsis et al., 2015) and as an endocrine organ (Pedersen and Febbraio, 2012).

The current consensus is that to diagnose sarcopenia, one should assess walking speed or grip strength and then examine appendicular lean mass if either is below a certain cutoff value (Cruz-Jentoft et al., 2010; Fielding et al., 2011; Morley et al., 2014). If muscle mass is lower than a healthy population cutoff value, sarcopenia is diagnosed. As such, low muscle mass continues to be an important component in the definition of sarcopenia. In 2016, the World Health Organization established an ICD-10 code for sarcopenia, which will spur the development of effective therapeutic strategies and increase the recognition of the importance of maintaining muscle mass and function with age for overall human health (Anker et al., 2016).

Resistance exercise continues to be the most effective intervention against sarcopenia (Landi et al., 2014). In addition, maintenance of physical activity can delay the progression of sarcopenia (Power et al., 2016a; Lazarus and Harridge, 2017). Despite the strong support for maintaining an active lifestyle, adherence to physical activity guidelines remains low. The traditional therapeutic focus of sarcopenia treatment is to target growth-related pathways to increase muscle mass. Here, we discuss the positives of these strategies, but also build a case for targeting mitochondrial bioenergetics as a way to maintain muscle mass and function with age as summarized in **Figure 1**. This review will cover the etiology of muscle loss, three basic characteristics of aging that may contribute to sarcopenia, current treatments targeting mass, and how targeting mitochondria rather than mass could mitigate basic mechanisms of aging to slow sarcopenia.

MECHANISMS LEADING TO LOSS OF STRENGTH AND FUNCTION

Etiology of Muscle Loss

The etiology of sarcopenia is characterized by both slow, gradual loss of muscle mass over time that is propagated by acute periods of accelerated loss and poor nutrition (English and Paddon-Jones, 2010). Acute periods of muscle loss in older individuals is often met with an incomplete regain of muscle mass and strength, thus accelerating gradual sarcopenic progression (Suetta et al., 2009; White et al., 2015; Baehr et al., 2016). The inability to completely regain muscle mass and strength is common in both aging humans (Suetta et al., 2009) and animals (White et al., 2015; Baehr et al., 2016). This public health problem is particularly troublesome for older adults who comprise the majority of hospital patients in the United States (DeFrances et al., 2008; Fisher et al., 2010) and who may lose more muscle mass during bed rest (Paddon-Jones et al., 2004; Kortebein et al., 2007). Older adults do not adequately recover following bed rest without adequate rehabilitation (Suetta et al., 2009, 2013; Hvid et al., 2010), which likely contributes to their reduced functional status and ambulation upon discharge (Covinsky et al., 2003; Coker et al., 2015). It is important to target both the gradual and accelerated periods of muscle loss to mitigate the progression of sarcopenia.

The vast majority of adults fail to meet physical activity guidelines. While 60% of adults, both European and American, self-report that they meet guidelines, objectively measured physical activity reveals that fewer than 10% of adults in the United States meet physical activity guidelines (Tucker et al., 2011; Marques et al., 2015). Moreover, sedentary behavior alone increases the risk for sarcopenia. While there are few trials in humans on the effects of lifelong sedentary behavior, studies in mice reveal lifelong sedentary behavior impairs mitochondrial function (Figueiredo et al., 2009). Moreover, a cross-sectional study in men revealed that sedentary behavior increased inflammation independent of physical activity (Parsons et al., 2017). Other lifestyle behaviors such as diet, combined with a sedentary lifestyle, also predispose individuals to increased risk for sarcopenia. An analysis of four prospective studies revealed that obesity increased the risk of developing sarcopenia by 20–162% (Stenholm et al., 2008). Indeed, sarcopenia and obesity often occur together, and evidence suggests that risk of metabolic disease and mortality increase when both are present (Wannamethee and Atkins, 2015). Obesity is characterized by inflammation and insulin resistance, both of which contribute to vascular dysfunction, as indicated by reduced endothelium-mediated vasodilation, impaired skeletal muscle perfusion, and decreased myofiber capillary density. Greater adipose infiltration into skeletal muscle is associated with the loss of skeletal muscle strength and torque (Goodpaster et al., 2001a). Lifestyle factors contribute to changes in the basic processes that lead to muscle loss.

Muscle mass is controlled by a dynamic balance of protein synthesis and degradation. A loss of muscle mass occurs when protein synthesis and degradation tip toward net degradation. Strategies to maintain muscle mass have focused on increasing protein synthesis since this is thought to be the more dynamic regulator of mass. Signaling through the mechanistic target of rapamycin (mTOR) pathway is the major regulator of protein synthesis in skeletal muscle (Bodine et al., 2001; Glass, 2005). The activation of mTOR results in activation of p70 S6K causing an increase in protein translation, and the inhibition of 4e-binding protein (4e-BP1), which is a negative regulator of the eukaryotic

translation initiation factor-4e (EIF-4e). The activation of these pathways stimulates growth processes, primarily of the myofibrillar proteins. However, a focus purely on growth may not maintain protein quality as discussed later in the review.

Mechanisms of Muscle Loss—Basic Processes

Loss of Proteostasis

Proteostasis is the maintenance of protein homeostasis that refers to the location, concentration, conformation, and turnover of individual proteins (Balch et al., 2008). Proteostasis is essential for whole body and tissue function. Loss of proteostasis leads to the accumulation of damaged proteins (Levine and Stadtman, 2001; Ayyadevara et al., 2016). In skeletal muscle, impaired proteostasis could lead to the decline in quantity and quality of contractile proteins because of accumulation of damage and non-enzymatic modifications of these proteins. One such non-enzymatic modification of proteins, advanced glycation end-products (AGEs) (Semba et al., 2010) increases in concentration with age including in skeletal muscle (Haus et al., 2007). Because of cross-bridge formations, modified proteins are resistant to breakdown and accumulate and may contribute to tissue dysfunction (Barreiro and Hussain, 2010; Drenth et al., 2016) and mobility disability (Sun et al., 2012).

Since the enzymatic capacity to repair protein damage is low, protein turnover is essential to maintain the skeletal muscle proteome. Increased protein turnover should improve proteostasis by degrading damaged proteins and resynthesizing new, functional proteins. Therefore, even in the absence of muscle growth, protein turnover is a beneficial adaptation for tissue health. Protein turnover is an energetically costly process with a large proportion of this demand coming from the step of translation. Protein synthesis requires 12–72 ATP molecules per amino acid synthesized, an additional four phosphates (either ATP or GTP) per bond, and an additional 1–2 ATPs per fold (Lynch and Marinov, 2015). The energetic cost of protein breakdown remains only partially understood. Protein degradation occurs primarily through protein ubiquitination and proteasome degradation, or *via* the autophagy-lysosomal system. Ubiquitination requires 2 ATPs per ubiquitin tag and subsequent proteasome-mediated degradation of them requires between 100 and 200 ATPs per protein (Lynch and Marinov, 2015). Lysosomal degradation requires four ATPs for every amino acid. Altogether, protein synthesis accounts for 20% of basal metabolism and protein breakdown comprises another 5–15% of basal metabolism (Rolfe and Brown, 1997). Given the energetic demands of protein turnover, a reliable source of energetic production is essential to maintain proteostasis.

Reactive Oxygen Species

The balance between oxidant production and scavenging is essential in maintaining redox homeostasis. With age, redox homeostasis declines and leads to the progressive oxidation of cellular components, including contractile proteins, which leads to skeletal muscle dysfunction (Lourenço dos Santos et al., 2015). Evidence from studies of human skeletal muscle indicates a positive correlation between age and markers of oxidative damage including lipid peroxidation, protein carbonyl content, and 8-oxo-deoxyguanosine (8-oxo-dG), a measure of DNA oxidation (Capel et al., 2005).

Age-related impairments in mitochondrial function result in increased production of reactive oxygen species (ROS), which increases oxidative stress and contributes to the loss of proteostasis. Mitochondrial dysfunction contributes to an imbalance of reducing equivalents such as NADH, which increases ROS formation and leads to the oxidation of cellular components. Loss of structural integrity of mitochondrial supercomplexes and membranes also leads to increased ROS production and associates with impaired skeletal muscle function (Genova and Lenaz, 2014). An increasingly oxidized environment not only increases oxidative damage to cellular components, it also increases the formation of AGEs. In models such as the whole body SOD1 KO mouse (Jang et al., 2010) and the muscle-specific SOD1 KO mouse (Sakellariou et al., 2018), there is an accelerated muscle aging phenotype that resembles muscle aging in humans. Conversely, targeting oxidative stress has also shown efficacy at preventing the sarcopenic phenotype in aged mice (Lee et al., 2010; Vays et al., 2014). Therefore, these models provide support for an indirect and direct role of oxidative stress in skeletal muscle aging.

Inflammation

Inflammation is a response to a cellular disruption (infection, damage, detection of "non-self") in an effort to facilitate a return to homeostasis. Chronic, low-grade inflammation occurs with aging due to immunosenescence and has been coined "inflammaging." Inflammaging contributes to chronic diseases including cardiovascular disease and sarcopenia (Ferrucci and Fabbri, 2018). Pro-inflammatory cytokines including interleukin-1 (IL-1), interleukin-6 (IL-6), c-reactive protein (CRP), and tumor necrosis factor (TNF) are characteristic of inflammaging and therefore have been proposed as markers of age and disease risk (Justice et al., 2018).

Several studies have associated circulating inflammatory cytokines with decreased skeletal muscle strength. The InCHIANTI study, a prospective study of mobility in older adults, demonstrated that elevated inflammatory markers predicted handgrip and leg strength (Schrager et al., 2007). A Danish cross-sectional study indicated that inflammatory marker TNF was inversely related with lean mass (Pedersen et al., 2003). Others have found that high IL-6 (>5pg/ml) and CRP (>6.1pg/ml) levels increased the risk of losing more than 40% of muscle strength by twofold to threefold (Schaap et al., 2006). Given the evidence from corroborating studies of aging, it is likely that inflammation contributes to the etiology of sarcopenia (Chung et al., 2009).

The mechanisms by which inflammation contributes to sarcopenia are not completely understood. In a study of older male subjects, increased levels of pro-inflammatory ceramides impaired anabolic signaling following an acute bout of resistance exercise (Rivas et al., 2012) meaning that inflammation may impair normal anabolic responses. In addition, high-fat diet

(HFD)-fed rodents and muscle cell culture models consistently link ceramide with exacerbated muscle atrophy or impaired anabolic signaling (Hyde et al., 2005; Roseno et al., 2015). There is also speculation that inflammatory factors such as prostaglandins impair skeletal muscle function by stimulating the generation of reactive oxygen species (ROS) (Chung et al., 2009). As an example, sphingomyelinase, an enzyme involved in the inflammatory response, stimulates the release of ROS that results in decreased contractile function as a consequence of chronic inflammation (Ferreira et al., 2010). More studies are needed to determine the mechanisms by which inflammation contributes to sarcopenia.

Interface of Mitochondria and Basic Aging Processes

The decline in skeletal muscle mitochondrial capacity with aging has been extensively studied as a contributor to slower walking speed (Coen et al., 2013; Santanasto et al., 2016; Gonzalez-Freire et al., 2018), fatigability (Santanasto et al., 2015), and sarcopenia (Joseph et al., 2012; Gouspillou et al., 2014). Evidence from the Baltimore Longitudinal Study of Aging indicates that skeletal muscle *ex vivo* mitochondrial respiration parallels decline *in vivo* oxidative capacity, cardiorespiratory fitness, and muscle strength (Gonzalez-Freire et al., 2018). While a number of cross-sectional human studies have demonstrated lower mitochondrial function with chronological age (Trounce et al., 1989; Boffoli et al., 1994; Tonkonogi et al., 2003; Short et al., 2005; Lanza et al., 2008; Porter et al., 2015a), several others have failed to observe these changes (Rasmussen et al., 2003; Hutter et al., 2007; Larsen et al., 2012b; Gouspillou et al., 2014; Gram et al., 2014). These inconsistent results may be partially due to variation in the approaches used to assess mitochondrial function. In addition, many studies of mitochondrial function in aging have not controlled for important covariates including participant physical activity levels (Boffoli et al., 1994) and adiposity (Short et al., 2005), which likely confound the relationship between mitochondrial capacity and age (Proctor et al., 1995; Hutter et al., 2007; Lanza et al., 2008; Safdar et al., 2010). For example, Distefano and colleagues demonstrated a strong, inverse correlation between age and mitochondrial function (Distefano et al., 2016). However, when controlling for fitness and adiposity, age only accounted for 1–6% of the variation observed in maximal ATP production. Regardless, there is some factor associated with the aging process that contributes to mitochondrial decline. The National Institute on Aging (NIA) recently funded the study of muscle, mobility, and aging (SOMMA), the goal of which is to determine the combination of muscle properties (energetics, autophagy, denervation, and oxidative stress) that most strongly predicts major mobility disability, declines in fitness, 400-m walking speed, and muscle mass.

Evidence from preclinical models indicates a close link between mitochondrial energetics and control of muscle mass. Release of pro-apoptotic factors (Max, 1972; Adhihetty et al., 2007), morphological alterations (fission, swelling), energy stress *via* reduced ATP (Romanello et al., 2010), and increased mitochondrial reactive oxygen species (ROS) emission (Adhihetty et al., 2007; Müller et al., 2007; Kavazis et al., 2009; Min et al., 2011) have all been reported during muscle atrophy in preclinical studies. In addition, for a given concentration of ADP, mitochondria from aged muscle generate more ROS than young counterparts (Holloway et al., 2018). Combined with the age-related decline in endogenous antioxidant activity, the increase in ROS emission leads to an increase in concentration of unscavenged ROS (Dai et al., 2014).

Mitochondrial ROS can depress protein synthesis by decreasing phosphorylation of 4e-BP1 and impairing mTOR assembly (Pham et al., 2000; Shenton et al., 2006; Zhang et al., 2009). Mitochondria-targeted antioxidant treatment in rodents supports a crucial role for mitochondrial ROS in mediating muscle atrophy (Min et al., 2011; Powers et al., 2011). Increased ROS production can also exacerbate muscle atrophy (Jang et al., 2010). Mitochondrial ROS stimulate proteolytic degradation pathways (autophagy and proteasome system) (Li et al., 2003; Aucello et al., 2009; McClung et al., 2009; Hussain et al., 2010) and energetic stress (reduced ATP production), which can activate the AMP kinase (AMPK)-FoxO3 pathways leading to increased expression of the ubiquitin-proteasome system and lysosome-autophagy system (Greer et al., 2007; Romanello et al., 2010). Taken together, multiple lines of compelling preclinical evidence implicate a central role for mitochondrial energetics in muscle atrophy. In addition, ROS react with and damage cellular components, including contractile proteins, which decreases proteome integrity and increases the demand for somatic maintenance.

In regard to inflammation, dysfunctional mitochondria exacerbate the detrimental effects of pro-inflammatory cytokines. Mitochondria release damage-associated molecular patterns (mito-DAMPs) that stimulate inflammatory pathways (Tezze et al., 2017). The mito-DAMPs further act on proteins such as NOD-like receptor protein 3 (NLRP3), which is involved in heart disease and aging (Salminen et al., 2012). Along with increased ROS release and pro-inflammatory signaling, dysfunctional mitochondria exacerbate inflammatory signaling that impair cellular integrity and proteostasis, which consequently leads to myocyte death (Chung et al., 2009).

Mitochondria are central to maintaining the energetic resources to preserve proteostasis. When there is a mismatch between the rate of ATP production and the demand for ATP, expendable cellular processes are sacrificed (Hou, 2013). There are three broad categories of cellular processes: metabolism, growth, and somatic maintenance (Hou et al., 2008). Processes that fall under metabolism are those that sustain life including energy production. Growth involves cellular expansion (Gregory, 2001), while somatic maintenance involves maintaining the quality of the soma (Shanley and Kirkwood, 2000). If the ability to provide ATP on demand is constrained, the cell makes trade-offs between growth and somatic maintenance since metabolism is maintained (Hou, 2013). Thus, impairment of mitochondrial function can in turn compromise proteostasis. In addition, it is conceivable, although never tested, that a focus on growth processes could compromise cellular

maintenance if not matched by increased energy-producing capabilities.

To illustrate the importance of maintaining mitochondria to mitigate the detrimental declines of basic cellular processes in sarcopenia, the neuromuscular junction (NMJ) provides an excellent example. Degradation of the NMJ leads to the denervation of muscle fibers (Jang and Van Remmen, 2011), which appears to contribute to decreased muscle size, strength, and endurance (Sundberg et al., 2018). With age, type II myofibers are more prone to denervation and are reinnervated with type I motor units (Kelly et al., 2017). Reinnervation of type I motor units leads to previously denervated type II myofiber to adopt a type I myofiber phenotype (Kelly et al., 2017). The change in innervation could contribute to the decline in skeletal muscle strength with age (Nilwik et al., 2013), as evident by the observation that older individuals have a greater proportion of type I myofibers along with lower strength (Frontera et al., 2000). Some evidence suggests that declines in NMJ quality precede the impairment of skeletal muscle function (Spendiff et al., 2016); however, more research is still needed in this area.

Oxidative damage of the NMJ promotes the loss of skeletal muscle proteostasis (Vasilaki et al., 2017). Recent data suggest that impaired redox signaling, rather than oxidative damage *per se*, drives denervation (McDonagh et al., 2016). Mitochondria in neurons of aged organisms appear to emit more ROS which can disrupt redox signaling by desensitizing redox sensors that are responsible for adaptation (McDonagh et al., 2016). With aging, there is decrease in the ability to activate redox-related signaling pathways, and this failure causes further oxidative damage in the neuron which promotes NMJ degradation (Vasilaki et al., 2006). Therefore, targeting mitochondrial function in the NMJ or areas surrounding the NMJ may be a mechanism to protect against age-related muscle loss.

NON-EXERCISE TREATMENTS OF SARCOPENIA

Treating sarcopenia has revolved around therapeutic interventions to mitigate the loss of muscle mass. These therapies, which include targeting members of the transforming growth factor β (TGF-β) superfamily, testosterone, selective androgen receptor modulators (SARMS), and growth hormone (GH), among others, are currently or have been tested at various phase clinical trials (Garber, 2016; Morley, 2016). In the following sections, we will discuss the mechanism in which these therapies target muscle loss, along with their efficacy to treat sarcopenia.

Growth and differentiation factor 8 (GDF8 or Myostatin), a member of the TGF-β superfamily, and activin A are powerful negative regulators of skeletal muscle growth (Lee, 2004). Myostatin and activin A signal through the activin type II receptor (ActRIIB), which leads to activation of Smad 2/3 transcription factors, translocation to the nucleus, and activation of target genes. Myostatin negatively regulates Akt signaling preventing protein synthesis and also interferes with myoblast differentiation into myotubes and may also impair muscle growth. A number of anti-myostatin antibodies have been developed and tested in humans, all of which increase lean mass (Becker et al., 2015; Woodhouse et al., 2016; Rooks et al., 2017) and some also showed improved physical function and strength (Becker et al., 2015; Rooks et al., 2017). It was recently demonstrated that inhibition of activin A in primates enhanced muscle growth (Latres et al., 2017). However, concerns remain around the functional benefits from inhibiting signaling through ActRIIB as in myostatin knockout mice, there is a loss of specific force (Amthor et al., 2007). Inhibiting myostatin has also been linked with reduced mitochondrial capacity of skeletal muscle, poor muscle endurance, and fatigability (Mouisel et al., 2014).

Low levels of circulating testosterone in older men (otherwise known as hypogonadism) are associated with reduced lean body mass, bone mineral density, and increased fat mass (Saad et al., 2017). Mechanisms of action for testosterone include increasing protein synthesis (Wolfe et al., 2000; Ferrando et al., 2003) *via* Akt/mTOR activation (White et al., 2013) and reduction in adipose stem cells and activation of satellite cell recruitment (Kovacheva et al., 2010). There is strong evidence from intervention studies that treatment with testosterone is effective in increasing lean mass and reducing fat mass (Kenny et al., 2010; Srinivas-Shankar et al., 2010; Kvorning et al., 2013). However, the efficacy of testosterone to improve muscle-specific strength and physical function is less clear (Snyder et al., 1999; Saad et al., 2017). The Testosterone Trial in Older Men showed that a year of testosterone treatment in 790 older men had no benefit with respect to fatigue or walking distance (Snyder et al., 2016). Conversely, others have shown that treatment with testosterone improved muscle function (leg extension, triceps extension, biceps curl) (Ferrando et al., 2002) and grip strength (Morley et al., 1993; Sih et al., 1997) in older men with hypogonadism. Whether testosterone influences skeletal muscle mitochondrial energetics is also not clear. Testosterone deficiency is associated with reduced myocellular metabolism and mitochondrial energetics (Traish et al., 2011) and preclinical work indicates that testosterone can induce mitochondrial biogenic signaling (Usui et al., 2014). However, testosterone treatment did not alter the activity of enzymes known to regulate mitochondrial biogenesis or markers of oxidative phosphorylation and lipid metabolism in the skeletal muscle of aging men with low testosterone (Petersson et al., 2014).

Growth hormone (GH) was also considered a therapeutic for sarcopenia. There is well-described decline in activity of the GH/insulin-like growth factor-1 (IGF-1) axis in older adults (Zadik et al., 1985). In 1990, it was shown that administration of human GH to older healthy individuals could increase lean mass and it was thought at the time that GH might be an effective antiaging therapy (Rudman et al., 1990). However, subsequent studies indicated that GH increased muscle mass but not strength (Kim and Morley, 2005) and unfavorable side effects were also reported, including gynecomastia and carpal tunnel syndrome, and treated patients were more likely to experience impaired fasting glucose (Liu et al., 2007). In a cross-sectional study of healthy adults, GH/IGF-1 level corresponded to muscle mitochondrial function (by ^{31}P MRS) (Makimura et al., 2011). However, 2 weeks of GH treatment

led to a reduction in mitochondrial genes involved in β-oxidation and oxidative phosphorylation (Sjögren et al., 2007). Many of the pharmacological targets explored to date are generally efficacious in improving muscle mass. However, and perhaps germane to the lack of therapeutic effectiveness, it is less clear whether mitochondrial energetics are improved with these options.

Manipulation of diet is another approach for the treatment of sarcopenia that has received a lot of attention. Post-feeding hyperaminoacidemia is a potent stimulator of skeletal muscle protein synthesis in young adults and is blunted in older adults following ingestion of amino acid mixture, suggesting skeletal muscle of older individuals is anabolic resistant (Volpi et al., 2000). It has been proposed that a greater dietary intake of essential amino acids could help older adults maintain skeletal muscle mass (Volpi et al., 2013). However, recent studies examining increased protein intake in older individuals do not show increased lean body mass (LBM) accretion (Bhasin et al., 2018), and that the amount and quality of protein intake are not associated with muscle mass or strength in older adults (Gingrich et al., 2017). Therefore, there remain questions over whether anabolic responses from protein and amino acid intake that are classically determined over the short-term translate to long-term preservation or improvements in muscle mass.

Increasing vitamin D levels, which is commonly reduced in older adults, through dietary supplementation results in improved muscle mass and chair-stand test time, a surrogate of muscle power in older adults (Bauer et al., 2015). Interestingly, improving the vitamin D status of adults that are deficient resulted in improved mitochondrial oxidative function in skeletal muscle (Sinha et al., 2013; Rana et al., 2014). This finding suggests that improvements in muscle health by vitamin D supplementation could at least be partly due to improvements in mitochondrial oxidative phosphorylation in older adults. A greater understanding of the independent roles of these dietary components on the regulation of muscle mass and function is needed along with a focus on how they also impact mitochondrial energetics.

As illustrated, despite substantial evidence linking mitochondrial energetics to the etiology of sarcopenia, pharmacological therapies to date have not focused on mitochondrial targets but rather pathways that mediate increases in muscle mass. There are some exceptions. For example, there is currently an ongoing clinical trial testing whether metformin, an AMPK activator, enhances the response to resistance exercise (Long et al., 2017). This proposed treatment has the potential to improve cellular metabolism and mitochondrial biogenesis to improve anabolic responsiveness. Other studies have examined the effects of exogenous antioxidants, such as vitamin C, on muscle quality and oxidative stress. Vitamin C supplementation improves muscle function in at least one model of aged muscle (Ryan et al., 2010), but vitamin C is known to inhibit the redox signaling that leads to positive mitochondrial responses (Gomez-Cabrera et al., 2008; Paulsen et al., 2014; Bruns et al., 2018). Meta-analyses investigating the efficacy of long-term vitamin C supplementation conclude that supplementation actually increases the risk for disease and mortality (Bjelakovic et al., 2012). Other strategies targeting antioxidant mechanisms are promising though. For example, studies using a purported Nrf2 activator that increases endogenous antioxidants produces lifespan in male mice (Strong et al., 2016) and enhances protein synthesis during aerobic exercise training (Bruns et al., 2018).

In sum, clinical therapies that use pharmaceutical and nutritional intervention that focus solely on muscle mass regulation have been met with limited success in various phase clinical trials. Additionally, these therapies appear to have little or no influence on mitochondrial energetics. Therefore, additional interventions are needed that help preserve mitochondrial function and muscle proteostasis in older adults. On the other hand, exercise has been shown to be an effective countermeasure to age-related muscle loss. Below, we focus on how exercise training affects mitochondrial function to support the notion that targeting mitochondrial function is essential in developing effective treatments to mitigate sarcopenia.

EXERCISE FOR THE TREATMENT OF SARCOPENIA

Physical Activity With Aging

Physical activity tends to decline with aging and results in difficulties in daily life activities and normal functioning (Westerterp, 2000). Data from the Health, Aging, and Body Composition (HABC) study showed that older adults who maintain higher levels of physical activity levels are protected against functional or mobility limitations in comparison to sedentary adults (Brach et al., 2004). There are several mechanisms in which maintaining physical activity throughout the lifespan protects against the onset of sarcopenia. Physical activity is a determinant of mitochondrial function, which likely is one mechanism that maintains skeletal muscle function (Larsen et al., 2012a; Tevald et al., 2014). Compared to sedentary counterparts, lifelong and masters athletes maintain skeletal muscle function concomitantly with mitochondrial function (Zampieri et al., 2014; Power et al., 2016b). Physical activity also attenuates the age-related increase in inflammation and oxidative stress, which, as highlighted, contribute to the loss of skeletal muscle mass and function (Mikkelsen et al., 2013).

Physical activity in mid-life appears to have a protective effect on physical function, as Patel et al. showed that older adults who engaged in higher levels of physical activity in mid-life performed better on the Short Physical Performance Battery (SPPB) and have reduced incidences of mobility disability than those less active in mid-life (Patel et al., 2006). Collectively, these results reveal a potential effect of exercise pre-habilitation to prevent the deleterious effects of aging and sedentary lifestyle on muscle function in older adults. The pre-habilitative effects of physical activity have led to clinical investigation into whether interventions later in life can prevent muscle dysfunction with aging. The Lifestyle Interventions and Independence for Elders (LIFE) study was designed to determine whether a physical activity intervention might confer significant physical benefits in older adults in comparison to those in a health education program. The intervention included ~150 min/wk of

moderate-intensity walking, along with strength, flexibility, and balance training (Fielding et al., 2011; Pahor et al., 2014). The 6-month intervention reduced major mobility disability in older adults that participated in the physical activity protocol, as well as a reduced risk of persistent mobility disability in comparison to those in a health education program (Pahor et al., 2014). Collectively, the results of the LIFE study concluded that moderate increases in physical activity levels, which likely improve cellular bioenergetics, blunt the detrimental effects of primary aging on physical function in older adults. In the following sections, we will describe how structured exercise interventions of different modalities (i.e., strength and aerobic exercise training) lead to improvements in skeletal muscle mitochondrial health in older adults.

Resistance Exercise

Resistance exercise remains a widely prescribed means to prevent, mitigate, and even reverse sarcopenia. The efficacy of resistance exercise to stimulate lean mass accrual and increases in strength is well documented (Koopman and van Loon, 2009). It was thought that resistance exercise training had little or no effect on mitochondrial biogenesis or function. However, recent studies have shown that resistance exercise training increases mitochondrial protein fractional synthesis rates (FSRs) (Wilkinson et al., 2008; Robinson et al., 2017) and improves mitochondrial function (Porter et al., 2015b; Robinson et al., 2017). Young adults engaged in a resistance exercise program showed increases in mitochondrial enzyme activity and respiration (Porter et al., 2015b). While the changes in mitochondrial respiration are modest in comparison to endurance exercise, improvements in *in vivo* PCr recovery rates and oxidative capacity appear comparable in older adults engaged in either exercise intervention (Jubrias et al., 2001). Similarly, using permeabilized myofibers, resistance exercise training increases state III and maximal oxidative phosphorylation capacity in older adults, which is accompanied by improvements in ADP sensitivity (Holloway et al., 2018). Therefore, while resistance exercise directly stimulates myofibrillar protein accrual to promote strength, it may also have positive effects on mitochondrial function and proteostasis.

Novel training regimens, such as power training, have been proposed to improve muscle function in older adults. Power training has received significant interest to prevent the sarcopenic phenotype, due to its targeting of type II myofibers, which are more prone to atrophy in older adults (Purves-Smith et al., 2014; St-Jean Pelletier et al., 2017). Indeed, clinical studies have shown that power training improves muscle function in older adults that is comparable or slightly greater than traditional resistance exercise training (Reid et al., 2008; Tschopp et al., 2011). Though preservation of mitochondrial function through physical activity is an important factor in the prevention of sarcopenia, the effects of power training on organelle bioenergetics is unclear.

While resistance exercise remains an effective intervention to combat sarcopenia, there are several considerations when prescribing resistance exercise training to older individuals. These concerns have led to alternative resistance training programs that focus on low load. It appears that low-load, high-repetition exercise is equally effective as high-load exercise in stimulating muscle hypertrophy in healthy individuals (Schoenfeld et al., 2017). Blood flow restricted resistance exercise is a second modification that may reduce the load necessary for positive adaptations. The premise is that a metabolic stress (blood flow restriction) may stimulate both mitochondrial and hypertrophy responses. Even with low load and number of repetitions, blood flow restricted exercise can positively influence motor unit activation and hypertrophy (Farup et al., 2015). More studies on blood flow restriction are necessary to determine if there are additional mitochondrial adaptations beyond traditional resistance exercise.

Aerobic Exercise

Aerobic exercise is generally not appreciated as a stimulator of hypertrophy; however, there is evidence that it can lead to muscle hypertrophy. In older adults, aerobic exercise training improves myofiber size and strength, as well as whole muscle size and strength (Harber et al., 2012). Therefore, aerobic exercise stimulates muscle growth either directly or indirectly.

Nearly half a century ago, Holloszy first documented that aerobic exercise increases mitochondrial content (Holloszy, 1967). Since then, research has consistently documented that aerobic exercise improves both mitochondrial content and function (Menshikova et al., 2006; Jacobs and Lundby, 2013; Zampieri et al., 2014). Aerobic exercise increases mitochondrial turnover since it increases both mitochondrial biogenesis (protein synthesis) (Wilkinson et al., 2008; Scalzo et al., 2014) and mitophagy (mitochondrial-specific autophagy) (Drake et al., 2015). The improvement in the rate of ATP production from aerobic exercise training suggests that more energy is available to maintain proteostasis. Additionally, improvement in mitochondrial efficiency (reduction in ROS generated per oxygen consumed or ATP generated) suggests that there is less oxidative stress and damage, which would in turn improve the quality of the proteome. In all, aerobic exercise mediated improvements in mitochondrial function likely protects against sarcopenia (Musci et al., 2017).

Enhanced mitochondrial content and function can sustain greater energetic flux and oxidation of substrates. Increased energetic flux can decrease the accumulation of lipotoxic intermediates that promote inflammation and oxidative stress (Goodpaster et al., 2001b; Coen and Goodpaster, 2012). Exercise-induced improvements in substrate flux thus decrease inflammation and oxidatively modified proteins (Corcoran et al., 2007; Fabbri et al., 2016). It has been shown that long-term aerobic exercise training in diabetic individuals can return mitochondrial function to that of a lean individual (Konopka et al., 2015). Importantly, aerobic exercise improves the ability of the muscle to provide energy on demand. Having energy on demand reduces compromises between growth and somatic maintenance that comes during periods of energy shortage. Thus, aerobic exercise improves the ability to maintain cellular integrity and adaptation to stress (Ristow and Schmeisser, 2014).

EXERCISE AS A DISCOVERY TOOL (MOTRPAC)

Exercise and physical activity enhance various aspects of human health and prevent metabolic and neurological diseases as well as cancer. In 2016, the National Institutes of Health (NIH) common fund dedicated $170 million to the molecular transducers of physical activity consortium (MoTrPAC). The goal is to identify the molecular networks in key tissues that are changed in response to acute and chronic exercise (Neufer et al., 2015). Elucidating how acute molecular responses to exercise integrate over time and improve health outcomes will unveil novel mechanisms contributing to health and disease processes and identify potential new therapeutic targets to aid in the prevention and treatment of disease. Older adults will be included in the study cohort and so the molecular networks that are altered in muscle with aging will be captured, in addition to the effects of exercise on mitochondrial and proteostasis molecular networks. From this study, there is enormous potential for us to further understand how endurance and resistance exercise improve mitochondrial function and muscle health in older adults.

FUTURE DIRECTIONS

Additional studies are needed to directly answer the question of whether treatments that target mitochondrial adaptations, such as aerobic exercise, are sufficient to maintain muscle mass with age. From these studies, the formulation of practical guidelines that refine recommendations of frequency, intensity, and duration of such activities should be derived. After establishing the appropriate evidence, it will be important to integrate important modulating factors such as medications (Robinson et al., 2009) and dietary factors (Smith et al., 2015) for long-term outcomes. Although there is strong preclinical and clinical support for targeting mitochondria with exercise, more studies are needed to directly support its efficacy to mitigate sarcopenia.

SUMMARY

If one considers a few of the primary underlying contributors to the loss of muscle mass with age, that is, loss of proteostasis, inflammation, and oxidative stress, it is apparent that mitochondria should be a target to mitigate sarcopenia (**Figure 1**). Unfortunately, to date, the field has primarily focused on directly stimulating growth processes rather than basic processes that create an environment that is favorable for growth processes. In other tissue types such as cardiac, hepatic, and nervous, it is well recognized that maintaining cellular energetics is of primary importance for maintaining tissue quality with age. Although skeletal muscle mitochondria are viewed as important for metabolism, the role of mitochondria in maintaining muscle mass is less appreciated. In our opinion, targeting mitochondria with exercise or other treatments is an effective way to treat sarcopenia and one that is still underexplored.

AUTHOR CONTRIBUTIONS

BM conceived of the review. PC, RM, MH, and BM wrote the review. PC and MH created the figure. PC, RM, MH, and BM proofread the review.

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Short-Term Exercise Training Inconsistently Influences Basal Testosterone in Older Men: A Systematic Review and Meta-Analysis

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Background: The age-associated decrease in testosterone is one mechanism suggested to accelerate the aging process in males. Therefore, approaches to increase endogenous testosterone may be of benefit. The aim of this paper was to undertake a Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA)-accordant meta-analysis concerning the effect of exercise on total (TT), bioavailable (bio-T), free (free-T), and salivary (sal-T) testosterone in older males.

Methods: Databases were searched up to and including 20th February 2018 for the terms "testosterone AND exercise AND aging AND males," "testosterone AND exercise AND old AND males," "testosterone AND training AND aging AND males," and "testosterone AND training AND old AND males". From 1259 originally identified titles, 22 studies (randomized controlled trials; RCTs; $n = 9$, and uncontrolled trials; UCTs; $n = 13$) were included which had a training component, participants ≥60 years of age, and salivary or serum testosterone as an outcome measure. Meta-analyses were conducted on change to testosterone following training using standardized difference in means (SDM) and random effects models.

Results: The overall SDM for endurance training, resistance training, and interval training was 0.398 (95% CI = 0.034–0.761; $P = 0.010$), −0.003 (95% CI = −0.330–0.324; $P = 0.986$), and 0.283 (95% CI = 0.030–0.535; $P = 0.028$), respectively. Resistance training exhibited a qualitative effect of hormone fraction whereby free-T resulted in the greatest SDM (0.253; 95% CI = −0.043–0.549; $P = 0.094$), followed by TT (0.028; 95% CI = −0.204–0.260; $P = 0.813$), and resistance training negatively influenced bio-T (−0.373; 95% CI = −0.789–0.042; $P = 0.078$). Due to the small number of studies, subgroup analysis was not possible for endurance training and interval training studies.

Conclusions: Data from the present investigation suggests that resistance training does not significantly influence basal testosterone in older men. Magnitude of effect

was influenced by hormone fraction, even within the same investigation. Aerobic training and interval training did result in small, significant increases in basal testosterone. The magnitude of effect is small but the existing data are encouraging and may be an avenue for further research.

Keywords: endurance, endocrine, exercise, HIIT, interval, resistance, testosterone, weight training

INTRODUCTION

Rationale

Muscle mass and function are particularly important in older adults, as epidemiological evidence suggests a positive relationship with longevity (Metter et al., 2004; Srikanthan and Karlamangla, 2014). Sarcopenia, defined as a loss of muscle mass coupled with functional deterioration, is now a clinically recognized disease deserving international attention (Cao and Morley, 2016). Given the increasing age of the world's population, maintaining muscle function into later life is imperative to avoid spiraling public health costs. Like muscle mass and function, serum testosterone typically declines with age (Tenover, 1997; Harman et al., 2001), and low testosterone is associated with many non-communicable diseases such as diabetes (El Baba and Azar, 2013; Mazur et al., 2014), cardiovascular disease (Schooling, 2014; Yeap, 2015), Alzheimer's disease (Lv et al., 2016), dementia (Carcaillon et al., 2014), obesity (Kelly and Jones, 2015), and ultimately mortality (Shores et al., 2012; Muraleedharan et al., 2013; Yeap, 2015). Several studies have reported improved health outcomes with exogenous testosterone administration, but side-effects are common, particularly cardiovascular events (Kim, 1999; Basaria et al., 2010; Yeap, 2015). Some effects of testosterone administration mimic those of exercise training. For example, Atkinson et al. (2010) reported testosterone administration preserved muscle mass in elderly individuals, which is the same effect appropriately prescribed exercise exerts (Frontera et al., 1988; Fiatarone et al., 1990; Herbert et al., 2017b).

In view of the complications with testosterone administration, exercise has been proposed as a non-pharmacological intervention to increase serum testosterone in older males (Swerdloff and Anawalt, 2014; Hayes et al., 2015d). However, the effect of exercise on testosterone is poorly defined, even within the same research group. For example, recent data suggest that although endurance-trained masters athletes and sedentary older adults exhibit similar total testosterone (TT), bioavailable testosterone (bio-T), and free testosterone (free-T) (Hayes et al., 2015b), salivary testosterone (sal-T) significantly differed between the trained and untrained older men (Hayes et al., 2013a). Further, although no difference in mean TT existed, more of the sedentary individuals were classed as biochemically hypogonadal (clinically low TT) than the masters athletes (Hayes et al., 2017b). In contrast, Cooper et al. (1998) noted masters endurance runners had greater TT concentrations (∼19 nmol·L^{-1} vs. ∼15 nmol·L^{-1}), but lower free androgen index (an estimate of biologically active testosterone; ∼21 vs. ∼31) than sedentary counterparts. However, the clinical significance of greater basal testosterone, within a "normal" physiological range, is unknown.

Different exercise modalities (i.e., endurance training, resistance training, interval training), and within-mode variables (i.e., intensity, volume, duration) may cause further discrepancies between investigations. Moreover, the portion of testosterone measured is not consistent between studies and can influence the direction and magnitude of response to exercise (Hayes et al., 2015d). For clarity, serum testosterone is mainly bound to sex hormone binding globulin (SHBG) and albumin. SHBG-bound testosterone is unavailable tissue uptake, whereas albumin-bound testosterone has access to target tissues because albumin-bound testosterone dissociates rapidly (Vermeulen et al., 1999). The non-SHBG-bound testosterone bound to albumin is therefore referred to as "bioavailable." The portion of testosterone completely unbound to SHBG or albumin is referred to as "free." TT encompasses SHBG-bound, bioavailable (albumin-bound), and unbound testosterone (i.e., 100% of that measured in the blood). Although these definitions are commonly used, there are numerous analytical methods for direct detection of testosterone, but also direct and indirect methods for quantifying bio-T and free-T. Different methods of analysis have dissimilar levels of variance and precision, which may conflate results. Furthermore, numerous exercise studies now measure testosterone in saliva (Keevil et al., 2017; Arruda et al., 2018; Chen et al., 2018) because of the ease of sample collection, despite methodological concerns (Hayes et al., 2015a,c, 2016). As such, the following normative values and clinical thresholds values cannot be applied to all laboratories, and must be interpreted with caution. Ranges for TT have been outlined as 10.4–32.6 nmol·L^{-1} (300–940 ng·dl^{-1}) for 30 year old males, 9.3–31.3 nmol·L^{-1} (268–903 ng·dl^{-1}) for 50 year old males, and 8.6–30.7 nmol·L^{-1} (248–885 ng·dl^{-1}) for 70 year old males (Bjerner et al., 2009), with Harman et al. (2001) suggesting a threshold of 11.3 nmol·L^{-1} (326 ng·dl^{-1}) for clinically low testosterone. However, Lazarou et al. (2006) reported that there were 17 different threshold values for TT to define hypogonadism across 25 laboratories. Indeed, the threshold for hypogonadism diagnosis varied by 350% (130 ng·dl^{-1}-450 ng·dl^{-1} (4.5–15.6 nmol·L^{-1}).

Objectives

Despite the potential of exercise training to increase testosterone in older males, there was no meta-analysis to provide pooled analysis of published studies to date. Therefore, the aim of this investigation was to conduct meta-analyses on the effect of aerobic, resistance, and interval training on TT, bio-T, free-T, and sal-T. A secondary aim was to investigate study characteristics

(i.e., research design and hormone fraction reported) on magnitude of effect.

METHODS

Eligibility Criteria

This meta-analysis was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines. Studies that met the following criteria were included: (1) published as a full-text manuscript; (2) not a review; (3) participants were apparently healthy older males (mean group age ≥60 years); (4) studies were required to employ an intervention design and include an exercise training period of >4 weeks. Additionally, descriptive data (e.g., sample size, mean, and standard deviation) were required to be reported. Where this was not possible, details were requested from authors. The primary aim was to investigate whether basal testosterone was affected by exercise training and therefore we only included studies that measured testosterone (TT, bio-T, free-T, or sal-T). Where an investigation took multiple measures, we included them as separate datasets.

Initially, this review aimed to consider randomized controlled trials (RCTs) and non-randomized control trials (CTs). However, due to the small number of RCTs and CTs, the study was extended to include uncontrolled trials (UCTs). For clarity, UCTs were analyzed separately from RCTs and CTs.

Information Sources

PubMed, ScienceDirect, and SPORTDiscus were searched with no start data, up until 20th February 2018. The search was performed within all fields and terms were "testosterone AND exercise AND aging AND males," "testosterone AND exercise AND old AND males," "testosterone AND training AND aging AND males," and "testosterone AND training AND old AND males."

Study Selection

Both authors conducted the eligibility assessment in an unblinded and standardized manner. Once each database search was completed and manuscripts were sourced, all studies were downloaded into a single reference list with duplicates removed. Titles and abstracts were then screened for eligibility and full texts were only retrieved for studies with testosterone and an exercise intervention incorporated. Two independent reviewers then read and coded all the included articles using the PEDro scale (Maher et al., 2003). Full texts were then thoroughly assessed using the complete eligibility criteria with first and second authors confirming inclusion and exclusion. Following this quality assessment, the same reviewers read and coded each of the studies and assessed the following moderators: design method (RCT or UCT), exercise type (endurance training, resistance training, or interval training), and hormone fraction (total, free, bioavailable, salivary). Furthermore, participant descriptions and training programme variables were extracted with as much detail provided by the authors. Any disagreement between both reviewers was discussed in a consensus meeting, and unresolved items were addressed by a third independent reviewer for resolution.

Data Collection Process

Data were extracted for pre- and post-training basal testosterone concentrations. In cases of missing data, authors were contacted via email and asked to provide necessary information. If no response was received, means and standard deviations (SDs) were estimated from figures using computer software (Image J, Maryland, USA, Imagej.net). Information was imported into a spreadsheet, which was specifically designed for meta-analyses (Comprehensive meta-analysis, NJ, USA).

Data Items

Heterogeneity was quantified with the I^2 statistic. An I^2 value of 25% may be interpreted as low, 50% as moderate and 75% as high between study heterogeneity. Three random-effect meta-analyses (endurance training, resistance training, and interval training) were conducted as each of these training types have different physiological demands and subsequent adaptations. Data extracted from each study included; study sample size, group descriptions, study design, analysis method, and outcome data. Furthermore, methodological quality was assessed using the modified 0–10 PEDro scale (de Morton, 2009). The primary outcome variables were defined as TT, bio-T, free-T, or sal-T pre- and post-intervention. Standard differences in means (SDM) were computed for the three meta-analyses by the software using the following equation (Higgins and Green, 2011):

$$\mathrm{SDM} = (\mu_1 - \mu_2) \div \sigma$$

Whereby: μ_1 = treatment mean, μ_2 = control mean, and σ = pooled standard deviation

Where the SD for change between time points (i.e., pre- and post-training change) was not reported, it was calculated thusly:

$$\sigma_{\mathrm{change}} = \sqrt{\left(\sigma_1^2 + \sigma_2^2 - (2 \cdot corr \cdot \sigma_1 \cdot \sigma_2)\right)}$$

Whereby: corr = correlation coefficient, a value that describes the relationship between baseline and final measurements over time. The correlation coefficient observed in our laboratory was 0.9 for TT pre-and post-intervention in a group of older males and therefore this was the value used for analysis.

Where a study utilized a UCT design (i.e., one group before and after training), the pre-training value was considered as μ_2 and the post-training value was considered as μ_1. Subgroup analyses were performed based on hormone fraction (i.e., TT, bio-T, free-T, or sal-T) where possible. Further analyses were performed based on research design as a means of investigating heterogeneous results.

RESULTS

Study Selection

After the initial database search, 1259 records were identified (see **Figure 1**). Once duplicates were removed, 985 titles and abstracts

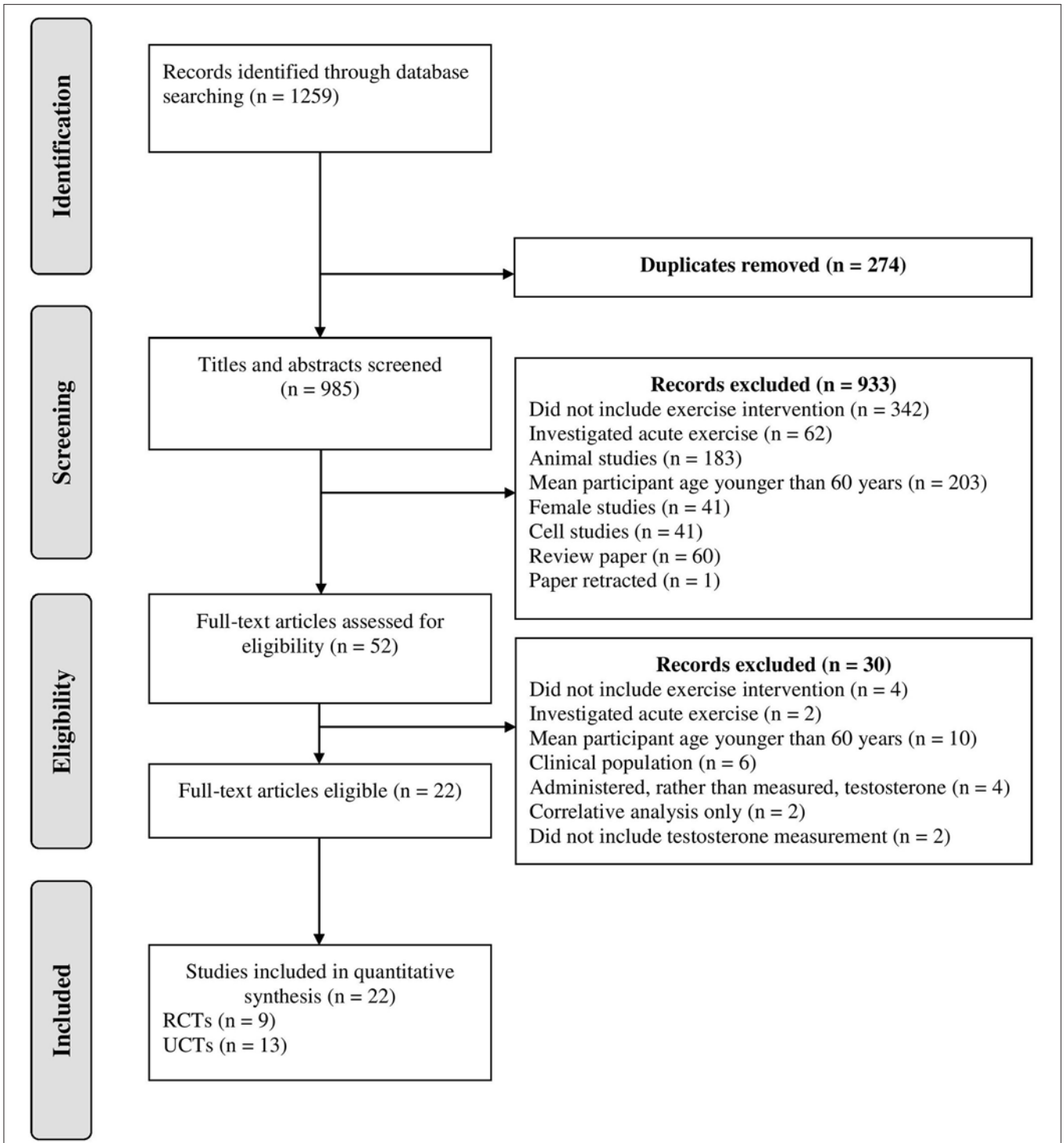

FIGURE 1 | Schematic flow diagram describing exclusions of potential studies and final number of studies. RCT, randomized control trial; UCT, uncontrolled trial.

were screened for inclusion by the authors resulting in 52 studies being retrieved as full text and assessed for eligibility. Of those, 30 were excluded and 22 articles remained and were used in the final quantitative synthesis. To assess publication bias, funnel plots for each exercise modality were computed and the trim and fill method was used (Duval and Tweedie, 2000). The trim and fill method determines the amount of studies required to eradicate publication bias from the funnel plot. For aerobic, resistance,

and interval training, the resultant number of imputed studies to eradicate bias was 0.

Study Characteristics

Of the 22 studies included, 9 were RCTs and 13 were UCTs (**Tables 1**–**3**). Where a study had multiple outcome measures (i.e., TT, bio-T, free-T, sal-T), they were treated as separate data points. Similarly, where a study reported values at multiple time points (i.e., 6 weeks, 12 weeks) they were treated separately.

Effect of Endurance Training on Testosterone

The overall SDM of endurance training was 0.398 (95% CI = 0.034–0.761; $P = 0.010$; **Figures 2**, **3**), and heterogeneity justified the use of a random effects model ($I^2 = 34.152$). Due to the small number of studies, the effect of hormone fraction and study design was not tested.

Effect of Resistance Training on Testosterone

The overall SDM of resistance training was −0.003 (95% CI = −0.330–0.324; $P = 0.986$; **Figures 4**, **5**) and heterogeneity justified the use of a random effects model ($I^2 = 37.340$). Qualitative and quantitative effects of hormone fraction were observed, whereby free-T resulted in the greatest SDM (0.253; 95% CI = −0.043–0.549; $P = 0.094$), followed by TT (0.028; 95% CI = −0.204–0.260; $P = 0.813$), and bio-T (-0.373; 95% CI = −0.789–0.042; $P = 0.631$). There was a qualitative effect of study design (i.e., the direction of the effect was influenced) whereby UCTs resulted in a greater SDM (0.205; 95% CI = −0.011–0.421; $P = 0.063$), than RCTs (−0.218; 95% CI =-0.481–0.045; $P = 0.105$).

Effect of Interval Training on Testosterone

The overall SDM of interval training was 0.283 (95% CI = 0.030–0.535; $P = 0.028$; **Figures 6**, **7**) with heterogeneity observed as $I^2 = 0.000$. Therefore, using a random or fixed effects model had no effect on the SDM or CIs, so for consistency we used a random effects model. Due to the small number of studies, the effect of hormone fraction and study design was not tested.

DISCUSSION

Overall SDM

The main finding from this meta-analysis was that short-term exercise training inconsistently influences basal testosterone in older men. The magnitude and statistical significance of effect varied with exercise modality, study design, and hormone fraction. Given that exercise training has been proposed as a first-line treatment for mild age-associated testosterone decrements (Swerdloff and Anawalt, 2014), this meta-analysis provides timely insight into the effect of exercise interventions on basal testosterone.

Endurance Training

The pooled effect of endurance training studies was a positive effect on basal testosterone in older males. Beside that of Lovell et al. (2012), studies displayed a positive SDM for endurance training. However, this finding of no change in response to training is a result of TT increasing similarly in the intervention group (∼1.1 nmol·L^{-1}) and in the control group (∼1.1 nmol·L^{-1}). What is interesting to note however, is the moderate increase in free-T in the same participants, following the same intervention. It would therefore seem reasonable to expect that a reduction in SHBG was responsible for this increased free fraction in the treatment group. In fact, SHBG increased less in the intervention group (∼1.0 nmol·L^{-1}) than in the control group (∼2.5 nmol·L^{-1}), which explains the moderate effect reported for free-T (treatment = ∼0.8 pmol·L^{-1}; control = ∼-1.4 pmol·L^{-1}). As such, it is postulated that biological or analytical variation likely caused this effect on free-T, which is supported by the large 95% CI. Similarly, Hayes et al. (2013b) reported a large increase in sal-T following aerobic conditioning, but sal-T

TABLE 1 | Description of included endurance training studies and data sets.

References	Exercise intervention	Design method	Outcome measures	Participants	PEDro score
Hayes et al., 2013b	Aerobic conditioning; 70–80% HR_{max}, 150 min·wk^{-1}, achieved by 2 d·wk^{-1} for 6 weeks	UCT vs. baseline	Salivary testosterone (ELISA)	$N = 28$ (age 63 ± 5, mass 90 ± 16 kg, stature 175 ± 3 cm). Lifelong sedentary.	3
Hayes et al., 2015d	Aerobic conditioning; 70–80% HR_{max}, 150 min·wk^{-1}, achieved by 2 d·wk^{-1} for 6 weeks	UCT vs. baseline	Total testosterone (ECLA) Bioavailable testosterone (Vermeulen equation) Free testosterone (Vermeulen equation)	$N = 28$ (age 63 ± 5, mass 90 ± 16 kg, stature 175 ± 3 cm). Lifelong sedentary.	3
Lovell et al., 2012	Periodized aerobic training; 2–3 d·wk^{-1} for 12 weeks.	RCT	Total testosterone (ECLA) Free testosterone (ECLA)	$N = 12$ (age 75 ± 3, mass 77 ± 12 kg, stature 177 ± 5 cm). Moderately active but without planned physical activity.	7
Struder et al., 1999	Periodized aerobic walking training; 5.6 km·h^{-1} for ∼30–60 min; 3 d·wk^{-1} for 20 weeks.	RCT	Total testosterone (EIA) Free testosterone (RIA)	$N = 11$ (age 69 ± 3, mass 76 ± 11 kg, stature 174 ± 8 cm). Sedentary.	7

HR_{max}, maximum heart rate; RCT, randomized control trial; UCT, uncontrolled trial; ECLA, electrochemiluminescence assay; EIA, enzyme immunoassay; ELISA, enzyme-linked immunosorbent assay. RIA, radioimmunoassay.

TABLE 2 | Description of included resistance training studies and data sets.

References	Exercise intervention	Design method	Outcome measures	Participants	PEDro score
Ahtiainen et al., 2011	1. Periodized resistance training; multi-set, multi-exercise, 40–90% 1-RM, ~2 d·wk^{-1} for 10 weeks. 2. Periodized resistance training; multi-set, multi-exercise, 40–90% 1-RM, ~2 d·wk^{-1} for 21 weeks.	RCT	Total testosterone (ECLA)	$N = 10$ (age 61 ± 5, mass 80 ± 5 kg, stature 177 ± 3 cm). Healthy but untrained.	7
Ahtiainen et al., 2015	Periodized resistance training; multi-set, multi-exercise, 40–90% 1-RM, 2 d·wk^{-1} for 12 months.	UCT vs. baseline	Total testosterone (ECLA) Free testosterone (Vermeulen equation)	$N = 9$ (age 70 ± 2, mass 80 ± 8 kg). Physically active but not resistance trained.	3
Armamento-Villareal et al., 2016	1. Multi-component training for 90 min; Aerobic: 65–85% HR_{max}, Resistance: multi-exercise, 65–85% 1-RM, 3 d·wk^{-1} for 6 months. 2. Multi-component training for 90 min; Aerobic: 65–85% HRmax, Resistance: multi-exercise, 65-85% 1-RM, 3 d·wk^{-1} for 12 months.	RCT	Total testosterone (automated immunoassay)	$N = 10$ (age 72 ± 2, mass 110 ± 3 kg). Frail, obese elderly.	6
Craig et al., 1989	Periodized resistance training; multi-set, multi-exercise, ~8–10-RM, 3 d·wk^{-1} for 12 weeks.	UCT vs. baseline	Total testosterone (RIA)	$N = 9$ (age 63 ± 1, mass 76 ± 2 kg). Physically active but not resistance trained.	3
Glintborg et al., 2013	1. Periodized resistance training; multi-set, multi-exercise, 2–3 d·wk^{-1} for 12 weeks. 2. Periodized resistance training; multi-set, multi-exercise, 2–3 d·wk^{-1} for 24 weeks.	RCT	Bioavailable testosterone (LC-MS/MS and Vermeulen equation)	$N = 16$ (age 68 [62–72]). Overweight men with low bioavailable testosterone. $N = 9$ (age 68 [62–72]). Overweight men with low bioavailable testosterone.	7
Glintborg et al., 2015	1. Progressive heavy strength training, 2–3 d·wk^{-1} for 3 months. 2. Progressive heavy strength training, 2–3 d·wk^{-1} for 6 months.	RCT	Bioavailable testosterone (LC-MS/MS and Vermeulen equation)	$N = 9$ (age 68 [62–72]). Overweight men with low bioavailable testosterone.	6
Hakkinen et al., 2002	1. Periodized heavy resistance training; multi-set, multi-exercise, 3-8-RM, 2 d·wk^{-1} for 12 weeks. 2. Periodized power training; multi-set, multi-exercise. 30–50% 1-RM, 2 d·wk^{-1} for 24 weeks.	RCT	Total testosterone (ELISA) Free testosterone (RIA)	$N = 10$ (age 65 ± 5, mass 84 ± 12 kg, stature 173 ± 7 cm). Healthy, mildly physically active.	7
Hakkinen and Pakarinen, 1994	Periodized resistance training; multi-set, multi-exercise, ~30–80% 1-RM, 2–3 d·wk^{-1} for 4 weeks. Periodized resistance training; multi-set, multi-exercise, ~30–80% 1-RM, 2–3 d·wk^{-1} for 8 weeks. Periodized resistance training; multi-set, multi-exercise, ~30–80% 1-RM, 2–3 d·wk^{-1} for 12 weeks.	UCT vs. baseline	Total testosterone (RIA) Free testosterone (RIA)	$N = 10$ (age 65 ± 1, mass 83 ± 6 kg, stature 171 ± 6 cm). Habitually active with no background in regular strength training.	3
Izquierdo et al., 2001	1. Periodized heavy and explosive resistance training; multi-set, multi-exercise, ~30–80% 1-RM. 2 d·wk^{-1} for 8 weeks. 2. Periodized heavy and explosive resistance training; multi-set, multi-exercise, ~30–80% 1-RM. 2 d·wk-1 for 16 weeks.	UCT vs. baseline	Total testosterone (RIA) Free testosterone (RIA)	$N = 11$ (age 64–73, mass 81 ± 10 kg, stature 167 ± 4 cm). Physically active but not resistance trained.	3
Katznelson et al., 2006	Theraband resistance training; multi-set, multi-exercise. 3–4 d·wk^{-1} for 12 weeks.	RCT	Total testosterone (RIA)	$N = 15$ (age 72 ± 6, mass 81 ± 14 kg). Ambulatory, community dwelling, sedentary men with serum free-testosterone <14.5 pg·ml^{-1}.	7

(Continued)

TABLE 2 | Continued

References	Exercise intervention	Design method	Outcome measures	Participants	PEDro score
Kraemer et al., 1999	1. Periodized resistance training; multi-set, multi-exercise, 3-15-RM, 3 d·wk^{-1} for 3 weeks. 2. Periodized resistance training; multi-set, multi-exercise, 3-15-RM, 3 d·wk^{-1} for 6 weeks. 3. Periodized resistance training; multi-set, multi-exercise, 3-15-RM, 3 d·wk^{-1} for 10 weeks.	UCT vs. baseline	Total testosterone (RIA) Free testosterone (RIA)	$N = 9$ (age 62 ± 3, mass 84 ± 13 kg, stature 174 ± 7 cm). Physically active but not resistance trained.	3
Kvorning et al., 2013	1. Periodized resistance training; multi-set, multi-exercise, 6-20-RM, 3 d·wk^{-1} for 12 weeks. 2. Periodized resistance training; multi-set, multi-exercise, 6-20-RM, 3 d·wk^{-1} for 24 weeks.	RCT	Bioavailable testosterone (LC-MS/MS and Vermeulen equation)	$N = 8$ (age 70 ± 2, mass 91 ± 1 kg, stature 178 ± 2 cm). Overweight men with low bioavailable testosterone.	7
Lovell et al., 2012	Periodized resistance training; multi-set, multi-exercise, ∼30–80% 1-RM, 2-3 d·wk^{-1} for 12 weeks.	RCT	Total testosterone (ECLA) Free testosterone (ECLA)	$N = 12$ (age 74 ± 3, mass 79 ± 14 kg, stature 178 ± 5 cm). Moderately active but without planned physical activity.	7
Petrella et al., 2006	Periodized resistance training; knee extensors, 8–12-RM, 3 d·wk^{-1} for 16 weeks.	UCT vs. baseline	Total testosterone (RIA) Free testosterone (Sodergard equation)	$N = 13$ (age 65 ± 1, mass 88 ± 3 kg, stature 179 ± 2 cm). Healthy but not resistance trained.	3
Sato et al., 2014	Periodized resistance training; knee extensors and flexors, 70% 1-RM, 3 d·wk^{-1} for 12 weeks.	UCT vs. baseline	Free testosterone (EIA)	$N = 13$ (age 67 ± 2, mass 64 ± 1 kg, stature 167 ± 1 cm). Moderately active but not resistance trained.	3
Vaczi et al., 2014	1. Periodized resistance training using eccentric contractions; knee extensors, 8–14 repetitions, 2-3 d·wk^{-1} for 10 weeks. 2. Periodized resistance training using stretch-shortening cycle; knee extensors, 8–14 repetitions, 2–3 d·wk^{-1} for 10 weeks.	UCT vs. baseline	Total testosterone (ECLA)	$N = 8$ (age 64 ± 4, mass 78 ± 12 kg, stature 182 ± 9 cm). Recreationally active but not resistance trained. $N = 8$ (age 66 ± 5, mass 79 ± 8 kg, stature 173 ± 6 cm). Recreationally active but not resistance trained.	3
Walker et al., 2015	Periodized resistance training; knee extensors and flexors, ∼8-14-RM, 2 d·wk^{-1} for 20 weeks.	UCT vs. baseline	Total testosterone (ECLA)	$N = 8$ (age 64 ± 3, mass 84 ± 7 kg, stature 177 ± 5 cm). Physically active but not resistance trained.	3

RM, repetition maximum; RCT, randomized control trial; UCT, uncontrolled trial; ECLA, electrochemiluminescence assay; RIA, radioimmunoassay; LC-MS/MS, liquid chromatography tandem mass spectrometry; ELISA, enzyme-linked immunosorbent assay; EIA, enzyme immunoassay.

is subject to large biological and analytical variation (Hayes et al., 2014), and this magnitude of effect was not the same in TT or free-T (Hayes et al., 2015d, 2017a).

Resistance Training

When all studies were pooled, resistance training had no effect on basal testosterone in older males. The largest negative effect for free-T was observed by Hakkinen et al. (2002). When comparing to similar duration interventions (Ahtiainen et al., 2015), and similar resistance training programmes (Hakkinen and Pakarinen, 1994; Kraemer et al., 1999; Kvorning et al., 2013), it is difficult to explain these results merely with time course or training variables. Moreover, the study exhibiting the largest positive effect on free-T (Kraemer et al., 1999) used a similar resistance training programme as Hakkinen et al. (2002), and the same detection method (radioimmunoassay [RIA]). As such, we suggest there is little effect of resistance training on any testosterone fraction in the aging male. This suggestion is supported by the studies with the largest (Kraemer et al., 1999) and smallest (Hakkinen et al., 2002) SDM not achieving statistical significance. When examining the results of Hakkinen et al. (2002) more closely, we propose the negative effect on TT was primarily a result of a trivial change in the intervention group, exaggerated by a trivial change of the opposite direction in the control group. The training group of Hakkinen et al. (2002) experienced no change to TT, yet the control group experienced an increase from ∼14.8 nmol·L^{-1} to ∼15.9 nmol·L^{-1} which resulted in an SDM of −0.269 for the intervention group over 24 weeks. Moreover, the increase in the control group represents a change of ∼13%, which is a trivial change using *post-hoc* analysis (Cohen's $d = 0.18$) and is within the critical difference outlined for TT (i.e., the

TABLE 3 | Description of included interval training studies and data sets.

References	Exercise intervention	Design method	Outcome measures	Participants	PEDro score
Hayes et al., 2017a	1. Aerobic preconditioning at 70–80% HR_{max} followed by HIIT described below. 150 min·wk^{-1}, achieved by 2 d·wk^{-1} for 6 weeks, then 6 weeks' HIIT 2. HIIT; 6 × 30 s @ 40% PPO, once every 5 days, for 6 weeks.	UCT vs. baseline UCT vs. preconditioning	Total testosterone (ECLA) Free testosterone (Vermeulen equation)	$N = 22$ (age 62 ± 2, mass 91 ± 16 kg, stature 175 ± 6 cm). Lifelong sedentary.	3
Herbert et al., 2017a	HIIT; 6 × 30 s @ 40% PPO, once every 5 days, for 6 weeks.	UCT vs. baseline	Total testosterone (ECLA) Free testosterone (Vermeulen equation)	$N = 17$ (age 60 ± 5, mass 78 ± 12 kg, stature 173 ± 6 cm). Masters athletes in endurance events.	3

HIIT, high intensity interval training; HRmax, maximum heart rate; PPO, peak power output; UCT, uncontrolled trial; ECLA, electrochemiluminescence assay.

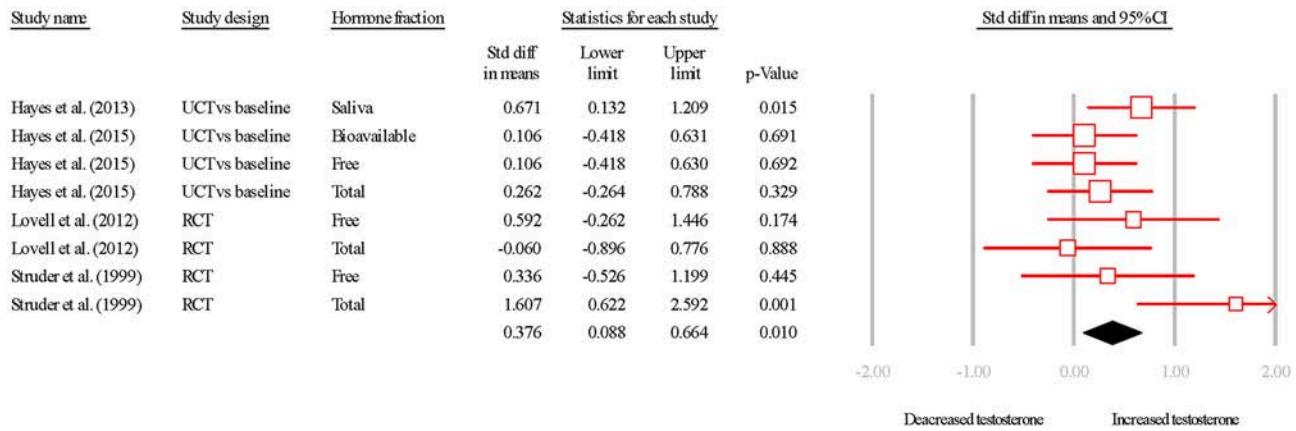

FIGURE 2 | Summary of studies examining aerobic exercise interventions on testosterone concentrations. 1,2,3 indicate separate conditions within one investigation. Filled diamond indicates overall SDM. SDM, standard difference in means; RCT, randomized controlled trial; UCT vs. baseline, uncontrolled trial; pre-intervention compared to post-intervention testosterone. Note that symbol size of individual studies is representative of the weighting for the pooled SDM.

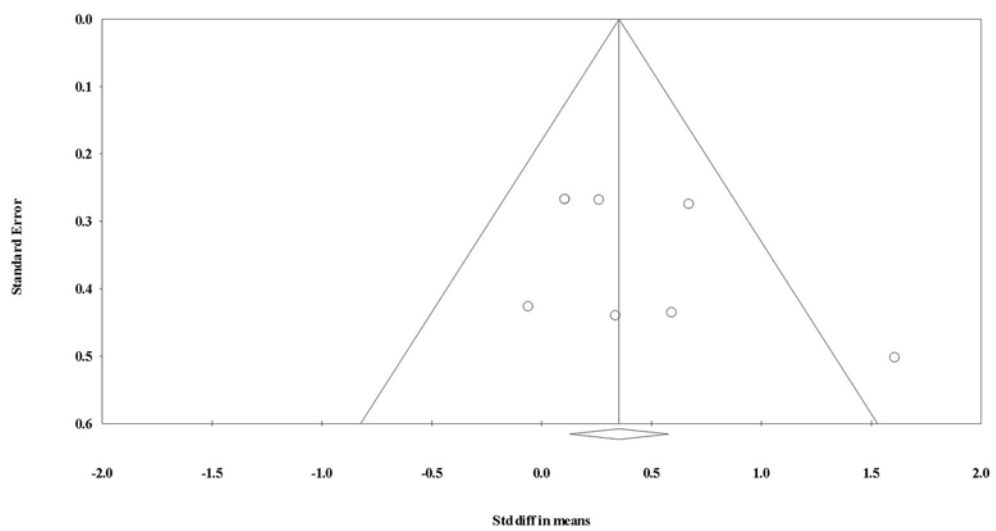

FIGURE 3 | Funnel plot for evaluating the effect of aerobic exercise on testosterone concentrations.

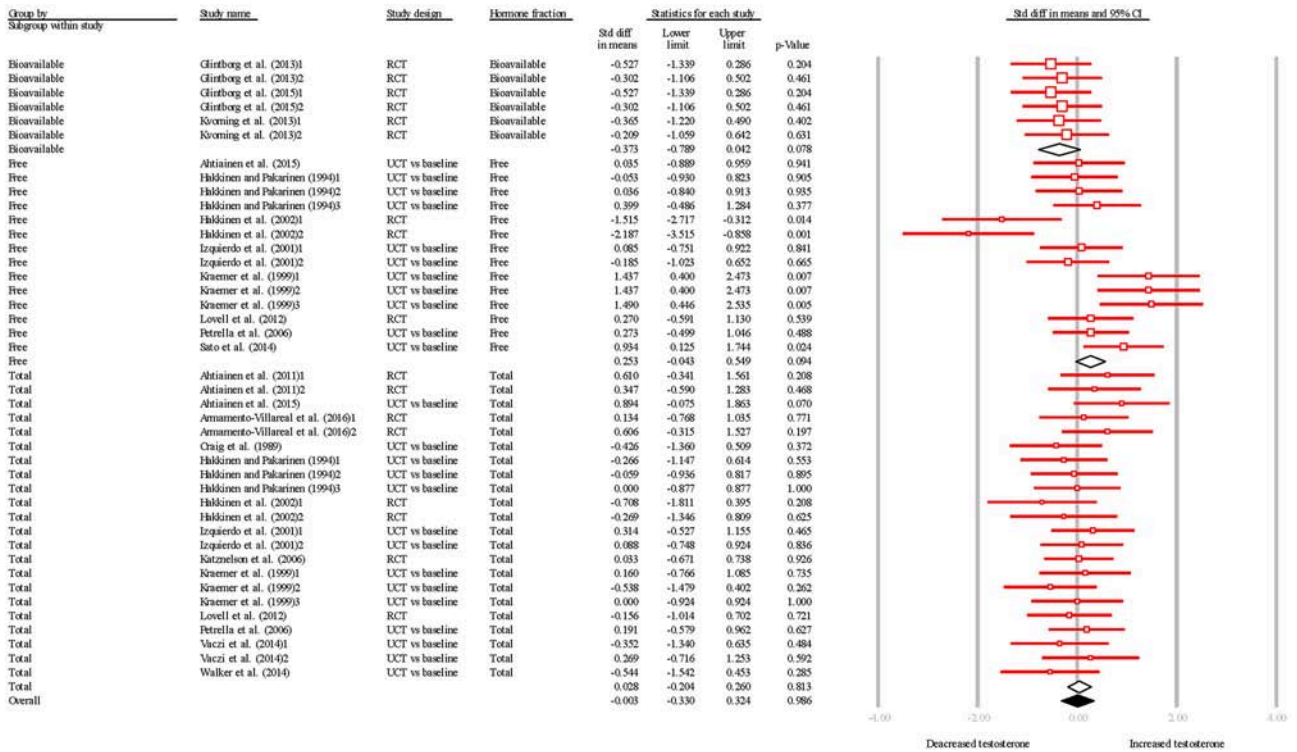

FIGURE 4 | Summary of studies examining resistance exercise interventions on testosterone concentrations. 1,2,3 indicate separate conditions within one investigation. Filled diamond indicates overall SDM. Empty diamond indicates pooled SDM for the hormone fraction. SDM, standard difference in means; RCT, randomized controlled trial; UCT vs. baseline, uncontrolled trial; pre-intervention compared to post-intervention testosterone. Note that symbol size of individual studies is representative of the weighting for the pooled SDM.

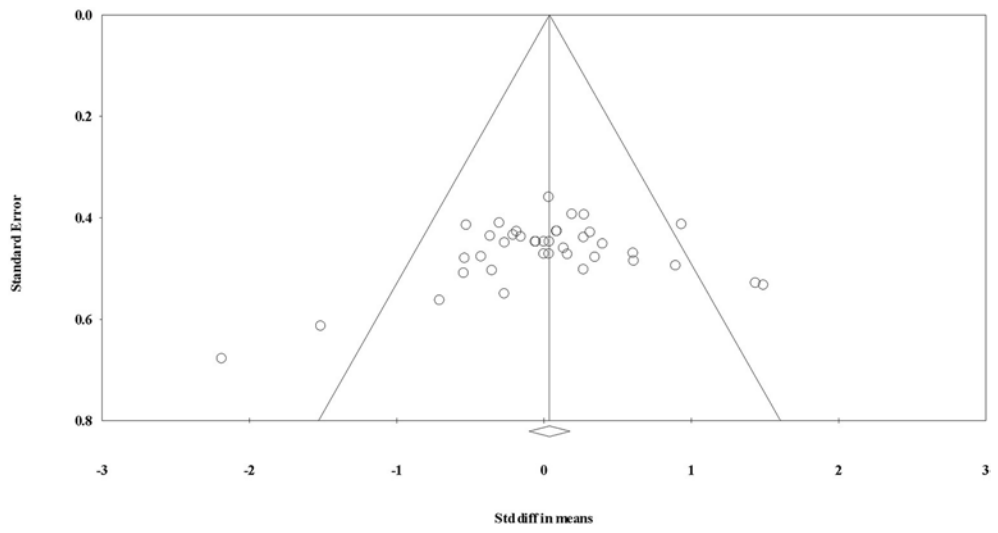

FIGURE 5 | Funnel plot for evaluating the effect of resistance exercise on testosterone concentrations.

threshold which needs to be exceeded for a change to be classed as biologically meaningful; Valero-Politi and Fuentes-Arderiu, 1993). Similarly, the training group experienced a ∼0.3 pmol·L^{-1} reduction in free-T, whereas the control group experienced a ∼14 pmol·L^{-1} increase, which resulted in an SDM of −2.187 over 24 weeks. However, the critical difference for free-T is yet to be established, and the increase of ∼33% may not be biologically meaningful.

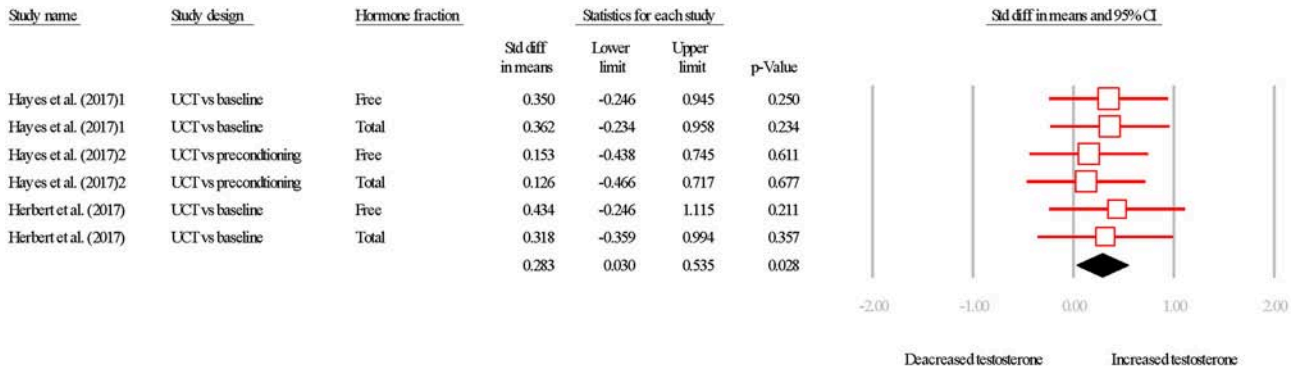

FIGURE 6 | Summary of studies examining interval exercise interventions on testosterone concentrations. 1,2,3 indicate separate conditions within one investigation. Filled diamond indicates overall SDM. SDM, standard difference in means; UCT vs. baseline, uncontrolled trial; pre-intervention compared to post-intervention testosterone. UCT vs. preconditioning, uncontrolled trial; post-intervention testosterone was compared to after a phase of- "aerobic preconditioning.' Note that symbol size of individual studies is representative of the weighting for the pooled SDM.

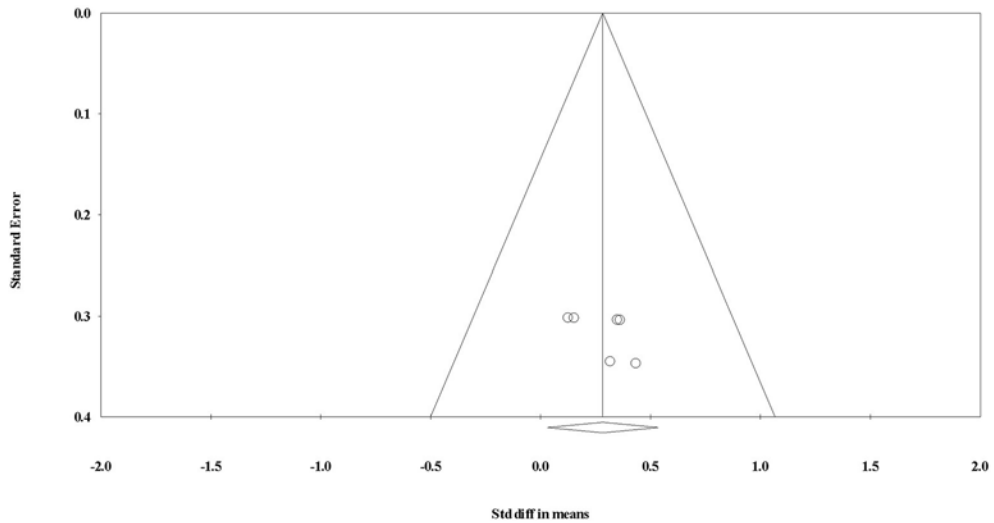

FIGURE 7 | Funnel plot for evaluating the effect of interval exercise on testosterone concentrations.

There were minor effects of sampling time on SDM. For example, Ahtiainen et al. (2011) observed a larger (yet still non-significant) change in free-T after 10 weeks, with a non-significant reduction from 10 weeks−21 weeks. An increase in androgen receptor (AR) expression could explain this minor decrease over time, as this would permit more testosterone-receptor interactions, which would remove free-T from circulation. However, no alteration to androgen receptor expression was observed by Ahtiainen et al. (2011). Further ambiguity is created by other studies reporting increased TT with longer time periods (Hakkinen et al., 2002). Again, we propose these changes are not biologically significant and are within the measurement error.

Whilst the effect of supraphysiological doses of testosterone on skeletal muscle are well-described (Bhasin et al., 2001; Sinha-Hikim et al., 2002; Deane et al., 2013; Hughes et al., 2016), the present investigation calls into question the importance of basal testosterone concentrations for increasing muscle mass in older men. Indeed, Vaczi et al. (2014) observed increased muscle strength and size despite clinically low testosterone in an older male population. Moreover, many investigations cited here reported increased muscle strength and size in the absence of increased basal testosterone (Hakkinen and Pakarinen, 1994; Izquierdo et al., 2001; Hakkinen et al., 2002; Ahtiainen et al., 2011).

Suppression of endogenous testosterone has been shown to attenuate muscle strength and mass gains in the young (Kvorning et al., 2006), and supraphysiological administration of testosterone increases muscle strength and mass (even without training) (Bhasin et al., 2001). As such, it appears that both low testosterone, and high testosterone, may be causal in muscular adaptations (or lack thereof). However, when testosterone is

within a "normal" physiological range, neither the acute elevation (West et al., 2010), nor the basal concentration appear to drive resistance training adaptations in an older population. This is supported by individuals with the lowest testosterone in this meta-analysis still experiencing increased muscle strength and mass. Increased AR expression, or testosterone-AR binding affinity could provide a mechanistic link between the *in vitro* data demonstrating testosterone's trophic effect on muscle, and increased muscle size without a concomitant increase in testosterone. However, further experimental investigation is necessary to confirm these speculations.

Interval Training

The two included interval training studies displayed a positive testosterone SDM, with both investigations coming from the same research group (Hayes et al., 2017a; Herbert et al., 2017a). This group used the same high intensity interval training (HIIT) in both studies, which consisted of 6 × 30 s sprints at 40% peak power output (∼130% peak oxygen uptake) interspersed with 3 min rest. Interestingly, both sedentary older males and endurance-trained masters athletes experienced increased free-T. As the magnitude of change was similar for free-T and TT in both investigations, this increase in free-T was likely driven by TT, rather than reductions in SHBG. These data are encouraging as HIIT has been promoted as a time-efficient method to improve cardiometabolic health (Gillen and Gibala, 2014; Cassidy et al., 2017; Phillips et al., 2017), and any androgenic improvement would be an additional benefit. However, caution must be exerted when (a) drawing conclusion from only two studies from one laboratory, and (b) implementing HIIT in older adults (Riebe et al., 2015). As such, further evidence is required to determine if the testosterone response to HIIT is consistent in older males, and whether HIIT is tolerable and safe in older populations.

Study Design

From the present meta-analysis, it is tempting to conclude RCTs result in different SDMs to UCTs. However, due to the distinct lack of RCTs it is difficult to justify such a conclusion, and further RCTs are warranted to add greater credence to the field. A further issue is the heterogeneity of exercise training design. When two studies used the same exercise intervention, for the same duration, free-T and TT responses were remarkably similar considering sedentary older men were compared to masters athletes (Hayes et al., 2017a; Herbert et al., 2017a). As such, it is possible the exercise intervention specifics (volume, intensity, frequency, etc.) may be more predictive of testosterone response to training that participant details or hormone fraction measured. However, without an investigation examining the testosterone response to two or more training programmes, matched for all variables except one (the dependent variable), this is purely speculation.

As with most exercise adaptations, individuals or groups with poorer starting values may be more susceptible to improvement. For example, untrained individuals commencing strength training should expect to increase their maximal strength more than experienced power lifters. Thus, it could be expected that individuals with low testosterone at enrolment would experience the greatest increase post-training. One investigation recruited individuals for their low testosterone (Kvorning et al., 2013), whilst three studies reported low mean starting TT <12 nmol·L^{-1} (Vaczi et al., 2014; Ahtiainen et al., 2015; Armamento-Villareal et al., 2016), and all included a resistance training intervention. Kvorning et al. (2013) observed a negative SDM for bio-T in aging men with low-normal testosterone, whilst Ahtiainen et al. (2015) reported no change to free-T but a positive change to TT. Armamento-Villareal et al. (2016) reported a substantial change in TT after 12 months but not after 6 months. As such, investigations recruiting biochemically hypogonadal individuals also report inconsistent findings, thus starting testosterone concentrations appears unlikely to influence the response to resistance training. Finally, Vaczi et al. (2014) reported no change to TT in individuals with TT ∼4 nmol·L^{-1}. However, ∼4 nmol·L^{-1} is in the very lowest range seen in our laboratory (Hayes et al., 2017a) and is classed as hypogonadal (Harman et al., 2001), and for that to be the mean value, an error in measurement or reporting may have occurred.

LIMITATIONS

The major limitation of the present meta-analysis is the lack of included studies, especially in aerobic and intervals training models, therefore a greater number of investigations would add weight to conclusions made herein. As such, conclusions made here are conservative and preliminary, until a greater depth of literature is available concerning exercise and basal testosterone in older males. Whilst the literature assessment was comprehensive, it is possible that studies may have been missed from the analysis, but as three databases were searched, it is unlikely enough were missed to create a large change to SDMs. Furthermore, having two authors ensured agreement on inclusion and exclusion, which limited potential bias.

To reduce heterogeneity, studies were classified into one of three broad exercise categories reflecting the physiological requirements of each training type. Yet, volume, intensity, and frequency of training cannot be controlled for. In fact, often it is difficult to discern the above acute programme variables within each study due to vagaries in reporting. For example, some investigations report % one-repetition maximum, whereas some authors report a number repetition maximum. Similarly, rest periods and number of sets are rarely reported in resistance training studies. It would improve the literature base if all authors adhered to the consensus on exercise reporting template (CERT; Slade et al., 2016) in future investigations. Whilst some investigations included have achieved statistical significance, the change to be considered biologically significant remains to be fully elucidated. Therefore, it is difficult to ascertain whether responses are clinically meaningful. While meta-analyses describe a population effect, i.e., group mean change, no investigations have reported whether individuals cross a clinical threshold (i.e., from hypogonadal to eugonadal), and therefore exercise as a treatment for low testosterone cannot be prescribed with confidence.

CONCLUSION

There is a pervasive belief that resistance exercise increases basal testosterone over time. However, that was not observed in the in older males in this meta-analysis. In fact, HIIT and endurance training showed the most promise for increasing basal testosterone in older men. There is a need for more RCTs to improve the quality of available evidence, as only seven studies in the present investigation achieved a score of 5 on the PEDro scale. The practical implication of this article is that resistance exercise may not be a viable solution to increase basal testosterone in the aging male, but aerobic and interval training may be. However, few studies examine whether exercise can raise testosterone from hypogonadal to eugonadal levels, which is of most clinical relevance and use for physicians.

Whilst here we report inconsistent effects of exercise training on basal testosterone in the eugonadal aging male, we do not argue that exercise training has a positive benefit in an aging population. Indeed, in many of the cited literature in this meta-analysis a lack of testosterone increase has not precluded physiological improvements following a training stimulus. Therefore, for older men, alterations in basal testosterone within the "normal" physiological range may not be mechanistically necessary for adaptation. Furthermore, testosterone is only one factor in the hormonal milieu, and exercise has been shown efficacious at improving other hormonal variables associated with successful aging and muscle function (Elliott et al., 2017; Sellami et al., 2017, 2018). Therefore, at present, exercise is probably our best non-pharmacological countermeasure to loss of muscle function with human aging.

AUTHOR CONTRIBUTIONS

LH and BE extracted and analyzed the data, drafted the manuscript, and proofed the manuscript.

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Normal Ribosomal Biogenesis but Shortened Protein Synthetic Response to Acute Eccentric Resistance Exercise in Old Skeletal Muscle

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Anabolic resistance to feeding in aged muscle is well-characterized; however, whether old skeletal muscle is intrinsically resistant to acute mechanical loading is less clear. The aim of this study was to determine the impact of aging on muscle protein synthesis (MPS), ribosome biogenesis, and protein breakdown in skeletal muscle following a single bout of resistance exercise. Adult male F344/BN rats aged 10 (Adult) and 30 (Old) months underwent unilateral maximal eccentric contractions of the hindlimb. Precursor rRNA increased early post-exercise (6–18 h), preceding elevations in ribosomal mass at 48 h in Adult and Old; there were no age-related differences in these responses. MPS increased early post-exercise in both Adult and Old; however, at 48 h of recovery, MPS returned to baseline in Old but not Adult. This abbreviated protein synthesis response in Old was associated with decreased levels of IRS1 protein and increased BiP, CHOP and eIF2α levels. Other than these responses, anabolic signaling was similar in Adult and Old muscle in the acute recovery phase. Basal proteasome activity was lower in Old, and resistance exercise did not increase the activity of either the ATP-dependent or independent proteasome, or autophagy (Cathepsin L activity) in either Adult or Old muscle. We conclude that MPS and ribosome biogenesis in response to maximal resistance exercise in old skeletal muscle are initially intact; however, the MPS response is abbreviated in Old, which may be the result of ER stress and/or blunted exercise-induced potentiation of the MPS response to feeding.

Keywords: ribosome biogenesis, ER stress, ubiquitin proteasome, IRS-1 signaling, anabolic resistance, sarcopenia

Abbreviations: BiP, endoplasmic reticulum binding protein; CHOP, CCAAT-enhancer-binding protein homologous protein; eIF2α, eukaryotic initation factor 2α; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; IRS1, insulin receptor substrate 1; ITS-1, internal transcribed spacer 1; MPB, muscle protein breakdown; MPS, muscle protein synthesis; mTORC1, mechanistic/mammalian target of rapamycin complex 1; PDK, phosphoinositide-dependent protein kinase; PI3K, phosphoinositide 3-OH kinase; rpS6, ribosomal protein S6; TAF1B, TATA box binding protein-associated factor RNA polymerase I B; UBF, upstream binding factor.

INTRODUCTION

Skeletal muscle anabolic resistance is a term that describes a reduced anabolic response to a given stimulus (for example: feeding, resistance exercise, or chronic loading), and is often used in an age-related context – i.e., old individuals exhibit anabolic resistance compared with young (Cuthbertson et al., 2005). Further, age-related loss in muscle mass appears to be underpinned by reduced responses to acute anabolic stimuli (Volpi et al., 2000) as opposed to lower basal rates of MPS (Volpi et al., 2001).

Age-related anabolic resistance to dietary protein feeding is relatively well-characterized (Volpi et al., 2000; Cuthbertson et al., 2005; Katsanos et al., 2005; Moore et al., 2015); by contrast, the effects of aging on the anabolic response to mechanical loading are poorly understood (Drummond et al., 2012; Baehr et al., 2016; Brook et al., 2016). Blunted rates of MPS and mTORC1 signaling in old individuals have been reported after moderate-to-high intensity resistance exercise (Kumar et al., 2009, 2012; Fry et al., 2011). However, observations of anabolic resistance to exercise may be confounded by underlying age-related differences in baseline activity/exercise habits (i.e., "active" old vs. sedentary old) (Burd et al., 2012b) and/or differences in motor unit activation during the exercise bout. Old individuals are reported to exhibit a reduced ability to drive their motor units at high muscle contraction intensities (Kamen et al., 1995). Thus, it is unclear from human studies the degree to which sarcopenia is the result of intrinsic and/or extrinsic factors – i.e., the contribution of the aging process *per se* vs. age-related reductions in habitual physical activity or motor unit activation (Clarke, 2004).

In well-controlled animal studies, age-related impairments in ribosome biogenesis and hypertrophy occur in response to synergist ablation (Hwee and Bodine, 2009; Kirby et al., 2015); however, synergist ablation increases muscle mass at a rate of 15–30% per week, whereas resistance exercise-induced gains are approximately 1–2% per week (Baar and Esser, 1999; Wernbom et al., 2007). Therefore, even though extreme models of muscle loading show impairments in ribosome biogenesis and hypertrophy with age, whether the response to physiological loading is impaired is unclear. In this study, we addressed this gap using a single bout of a well-established animal model of eccentrically biased resistance exercise (Wong and Booth, 1990; Baar and Esser, 1999; Chen et al., 2002; Hamilton et al., 2010; West et al., 2016). A detailed evaluation of this and other skeletal muscle hypertrophy models is beyond the scope of this paper; for more discussion on this topic readers are referred to reviews elsewhere (Timson, 1990; Cholewa et al., 2014).

The anabolic response to resistance exercise is characterized by alterations in skeletal muscle translational activity and capacity (Henshaw et al., 1971; Stec et al., 2015; West et al., 2016). Therefore, the primary purpose of the present study was to determine whether exercise-induced increases in translational activity and capacity are impacted by age after an acute bout of eccentric exercise using a model of maximal motor unit activation. To achieve this aim, we determined MPS, ribosome biogenesis, and acute mTORC1 signaling in recovery (6, 18, 48 h) from maximal resistance exercise in adult and old animals.

Even though much of the focus for declining muscle mass has concentrated on the protein synthesis side of the equation, age-related declines in autophagy and proteasome function may reduce clearance of dysfunctional proteins and contribute to cellular senescence (Anvar et al., 2011; Lopez-Otin et al., 2013; Hwee et al., 2014; Hentila et al., 2018). A senescent cell phenotype in skeletal muscle may impact the growth response to anabolic stimuli. Conversely, in the compensatory hypertrophy model, large increases in ubiquitin proteasome pathway (UPP) activity accompany increases in MPS and muscle mass (Baehr et al., 2014). These data suggest that increased proteasome activity is needed for chronic growth. Additionally, MuRF1 (an E3 ligase in the UPP) knockout animals exhibit elevated proteasome activity throughout their lifespan and this was associated with protection against age-related loss of muscle mass as well as improved growth in response to overload (Hwee et al., 2014). Taken together, proteasome function may be decreased in aging skeletal muscle and this may contribute to the impaired response to anabolic stimuli. Therefore, a second aim of this study was to investigate age-related differences in protein degradation pathways after resistance exercise.

MATERIALS AND METHODS

Animals and Exercise Protocol

Adult (10 months) and old (30 months) male Fischer 344-Brown Norway rats (**Table 1**) were used according to a protocol approved by the University of California Davis Animal Care and Use Committee. Animals were anesthetized (isoflurane inhalation, 2.5%) before undergoing acute unilateral electrical stimulation of the sciatic nerve to activate the hindlimb muscles. In this model of resistance exercise, the muscles in the anterior compartment (tibialis anterior; extensor digitorum longus) undergo high-force lengthening contractions as a result of the stronger antagonist muscles in the posterior compartment (gastrocnemius; plantaris, soleus) (Wong and Booth, 1990). After

TABLE 1 | Baseline body weight, hindlimb muscle weights and total RNA concentration in adult and old.

	Adult (10 months)	Old (30 months)	Old relative to adult
Body weight (g)	447.1	544.9	+21.9% $P < 0.001$
Muscle weight (g)			
TA	0.812 ± 0.054	0.712 ± 0.063	−12.4% $P = 0.001$
EDL	0.195 ± 0.011	0.181 ± 0.010	−7.2% $P = 0.009$
SOL	0.199 ± 0.019	0.179 ± 0.016	−9.9% $P = 0.021$
PLN	0.438 ± 0.034	0.365 ± 0.026	−16.7% $P < 0.001$
Total RNA (μg RNA/mg wet tissue)	0.800 ± 0.028	0.857 ± 0.057	+7.1% $P < 0.001$

Values are means ± SD. TA, tibialis anterior; EDL, extensor digitorum longus; SOL, soleus; PLN, plantaris.

stimulation, animals were placed back in their cages and allowed *ad libitum* access to food. Thirty minutes before muscle collections, animals were administered puromycin (0.02 μmol g^{-1} body weight by I.P. injection) for the determination of protein synthesis via the surface-sensing of translation method (Goodman et al., 2011). Animals were then anesthetized and hindlimb muscles were surgically removed and frozen in liquid nitrogen 6, 18, or 48 h following stimulation. The tibialis anterior muscle, which is a predominantly fast-twitch muscle, was powdered with a liquid nitrogen-cooled mortar and pestle before further analysis.

Protein Levels

Levels of select proteins, as well as puromycin integration, were measured by western blot. Briefly, an aliquot of frozen tissue powder was homogenized in a sucrose lysis buffer (50 mM Tris pH 7.5, 250 mM sucrose, 1 mM EDTA, 1 mM EGTA, 1% Triton X100, 50 mM NaF, 1 mM NaVO4 $Na_2(PO_4)_2$, and 0.1% DTT), centrifuged (10,000 *g* × 10 min), and the supernatant collected for protein concentration measurement (DC protein assay, Bio-Rad, cat. 500-0116). Protein concentrations were equilibrated, combined with Laemmli sample buffer, and heated at 95°C for 5 min. Gradient polyacrylamide gels (4–20%) containing non-exercised and exercised samples in adjacent wells were used to separate proteins by electrophoresis (200 V for 45 min), transferred to nitrocellulose membrane (wet transfer, 100 V for 1 h), blocked in 1% fish skin gelatin, and washed in Tris-buffered saline 0.1% tween (TBST) before overnight incubation in primary antibody (1:1000 in TBST) at 4°C. Primary antibodies were from: Millipore – puromycin (cat. MABE343); Cell Signaling Technologies (Danvers, MA, United States) – BiP (cat. 3183), CHOP (cat. 5554), phospho-eEF2 Thr56 (cat. 2331), phospho-eIF2α Ser51 (cat. 9721S), IRS1 (cat. 2382S), phospho-rpS6 Ser240/244 (cat.2215), phospho-S6K1 Thr389 (cat. 9205); and Santa Cruz Biotechnology (Santa Cruz, CA, United States) – phospho-UBF Ser637 (cat. 21639). Membranes were incubated in secondary antibody (1:10,000 in TBST; 1 h at RT) before protein expression detection by chemiluminescence (Millipore, cat. WBKLS0500). Blots were normalized for protein loading via Ponceau stain (**Figure 1B**). Images for densitometry analysis were captured with a Bio-Rad ChemiDoc MP imaging system and quantified using Image Lab Software (Bio-Rad, v. 5).

Total RNA and Gene Expression

Total RNA was extracted, from a pre-weighed aliquot of frozen TA muscle powder, using RNAzol RT (Sigma, cat. R4533) according to manufacturer's instructions. Absorbance was quantified by spectrophotometry (Epoch Microplate Spectrophotometer, BioTek Instruments Inc.). One microgram of RNA was converted to cDNA using a reverse transcription kit (Life Technologies, cat. 4368814) according to manufacturer's instructions. cDNA was diluted 10-fold before analysis by quantitative RT-PCR. Gene expression was calculated using the delta delta threshold cycle method (Livak and Schmittgen, 2001) (experimental vs. contralateral control) and GAPDH was used as a housekeeping gene. There was no difference in the absolute C_T of GAPDH as a result of either age (Adult = 15.104 +/− 0.410; Old = 15.192 +/− 0.381) or exercise (Adult = 15.219 +/− 0.407; Old = 15.110 +/− 0.320). Primer sequences are shown in **Table 2**.

Proteasome and Cathepsin L Activity Assays

Proteasome and cathepsin L activity assays were performed as previously described (Gomes et al., 2012). Briefly, frozen muscle powder was dounce-homogenized in cold buffer (50 mM Tris pH 7.5, 1 mM EDTA, 150 mM NaCl, 5 mM $MgCl_2$, 0.5 mM DTT) before centrifugation (12,000 g × 30 min at 4°C) and collection of the supernatant. Supernatant protein concentrations were determined and equal protein quantities were assayed: 8–20 μg/well for proteasomal subunit assays, and 34 μg/well for cathepsin L assays. Samples from REx and contralateral control legs, as well as adult and old groups, were run on the same plate. Fluorescence of tagged substrates (proteasome: 20–100 μM Leu-Leu-Val-Tyr—4-amino-7-methyl coumarin, BACHEM, cat. I–1395; cathepsin L, Z-Phe-Arg-MCA, Peptide Instituted Inc, Code: 3095-v) was measured kinetically (Fluoroskan Ascent 2.5, Thermo Electron, Waltham, MA, United States) to ensure assay linearity. Activity was determined by calculating the difference between wells with and without inhibitor (proteasome: Bortezomib, 2–10 μM; Calbiochem cat. 504314; cathepsin L: cathepsin L inhibitor I: 10 μM Calbiochem, cat. 219421).

Statistical Analysis

Two-way ANOVAs were used to analyse time course data (age × time) and proteasome data (age × condition) with Tukey's *post hoc*. Factors (levels) were as follows: age (Adult vs. Old), time (6, 18, and 48 h), and condition (experimental vs. control leg). Data was log transformed where appropriate to correct skewness and unequal variance before statistical analyses. Statistical analyses were performed using SigmaStat software (v.3.1, Systat Software, San Jose, CA, United States); statistical significance was set at $P < 0.05$.

TABLE 2 | 5′ to 3′ sequences of primers used for quantitative RT-PCR.

Gene	Forward	Reverse
Rat		
c-Myc	CAGCAGCGACTCT GAAGAAGAAC	GATGACCCTGA CTCGGACCTC
Glyceraldehyde 3-phosphate dehydrogenase	GTCATCCCA GAGCTGAACGG	GCCTGCTTC ACCACCTTCT
Internal transcribed spacer 1	TCCGTTTTCT CGCTCTTCCC	CCGGAGAGAT CACGTACCAC
Nucleolin	AAAGTGCCCC AGAACCCACA	TGGCTGACTTC TCGCATTAGG
Nucleophosmin	TGTCCAGGTTC AATTGCCAAG	CCAAGTAAAG GGCGGAGTT
TATA box binding protein-assoc. factor RNA Pol I B	CATCTTTGCTGT CGAGTCTTGG	GGATGGAGGTA GCAGTCTTCAG

RESULTS

Muscle Protein Synthesis and Ribosome Biogenesis

Muscle protein synthesis was moderately elevated (18–33%) after resistance exercise, and this response was more sustained in adult animals (Adult > Old at 48 h, $P = 0.046$; **Figure 1A**). Precursor ribosomal RNA expression was elevated at 18 h compared with 6 h ($P < 0.05$) and by 48 h precursor rRNA was in decline but not yet back to baseline (**Figure 1C**). Total RNA was elevated in Adult (23%, experimental/contralateral control) and Old (19%) at 48 h after exercise (**Figure 1D**), but was not different between age groups ($P = 0.38$). Total RNA at 48 h was elevated compared with 6 and 18 h after exercise (main effect of time, both $P < 0.001$). In control legs, total RNA concentration (μg RNA/mg wet tissue) was modestly (+7% in Old) but significantly ($P < 0.001$) higher in Old compared with Adult (**Table 1**). Hindlimb muscle weights of the 10 and 30 month old animals are shown in **Table 1**.

mTORC1 Signaling

The phosphorylation of S6K1^{Thr389} and ribosomal protein S6$^{Ser240/244}$ in response to exercise peaked at 6 h, declined at 18 h and returned to baseline levels at 48 h (**Figures 2A,B**). Elongation factor 2^{Thr56} phosphorylation decreased (**Figure 2C**), and UBF^{Ser637} (**Figure 2D**) phosphorylation increased following exercise. All of these responses were similar in Adult and Old muscles across the recovery time course.

Gene Expression

c-Myc expression was elevated by exercise in Adult and Old at 6 and 18 h, with no effect of age (**Figure 3A**). Nucleolin and nucleophosmin, myc target genes that play a role in precursor rRNA splicing, showed very similar (to each other) patterns of expression, and were both greater at 18 h of recovery in Old vs. Adult ($P = 0.034$ and 0.025 for nucleolin and nuclophosmin, respectively, **Figures 3B,D**). TATA box binding protein-associated factor RNA Pol I B (TAF1B), a component of the pre-initiation complex that mediates Pol I transcription of rDNA, tended to gradually rise to a "peak" at 48 h in Old, whereas TAF1B expression peaked at 6 h in Adult before declining at later time points ($P = 0.056$ for age × time interaction; **Figure 3C**).

ER Stress

Protein levels of the adaptive BiP were elevated by resistance exercise at 48 h in both Adult and Old (time effect, $P < 0.001$; **Figure 4A**), whereas CHOP was unchanged by resistance exercise but was constitutively higher in Old ($P < 0.001$; **Figure 4B**). Phospho-eIF2α Ser51 was elevated by resistance exercise at 18 h in Old but not Adult (**Figure 4C**).

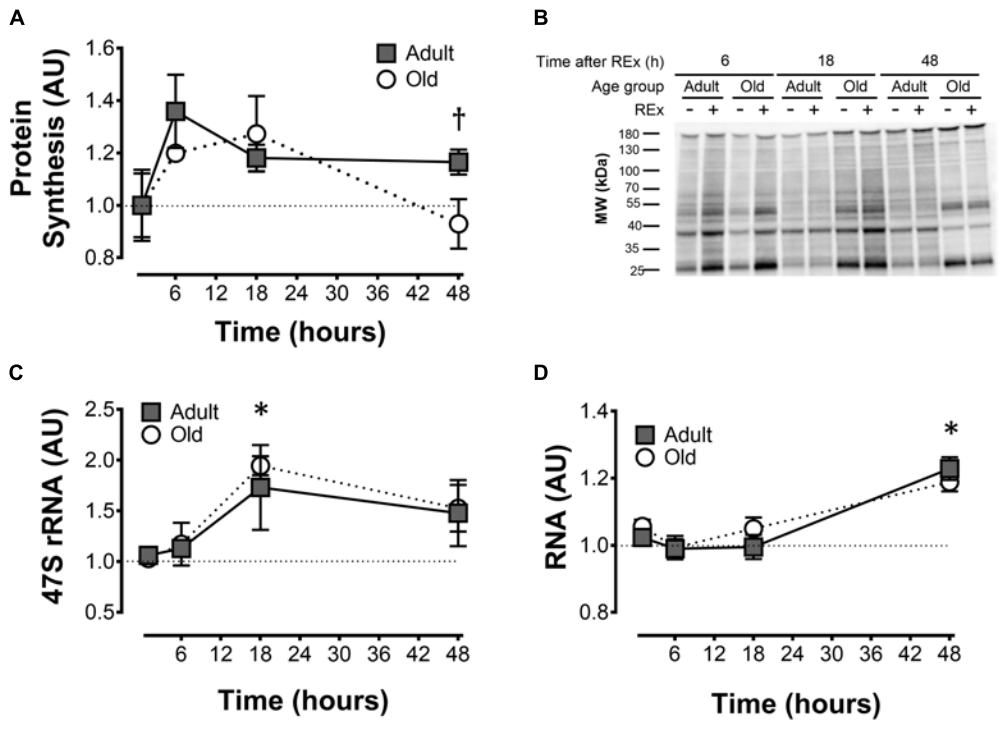

FIGURE 1 | Muscle protein synthesis and ribosome biogenesis after acute unilateral resistance exercise in adult (10 months) and old (30 months) rat skeletal muscle. Muscle protein synthesis **(A)** with representative blot showing puromycin incorporation **(B)**; 47S rRNA **(C)**, and total RNA **(D)**. †Difference between Adult and Old at the same time point, $P < 0.05$. *Main effect of time; different from 0 to 6 h, $P < 0.05$. Values are expressed as experimental/contralateral control muscles, means ± SEM; $n = 6$/group.

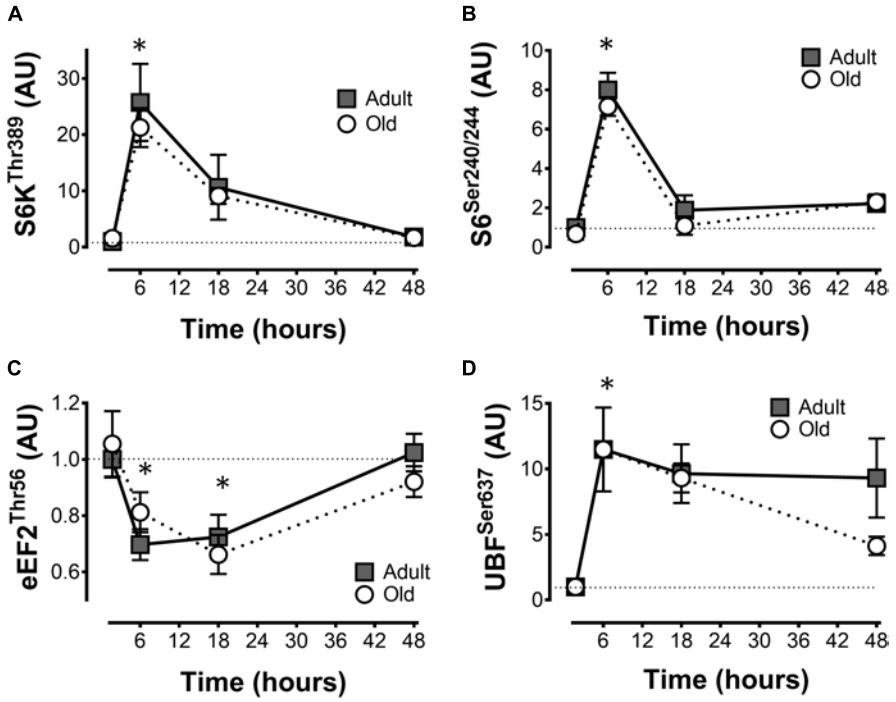

FIGURE 2 | mTORC1 signaling after acute unilateral resistance exercise in adult (10 months) and old (30 months) rat skeletal muscle. Ribosomal S6 kinase **(A)**, ribosomal protein S6 **(B)**, eukaryotic elongation factor 2 **(C)**, and upstream binding factor **(D)** phospho-protein levels. *Main effect of time; different from non-stimulated control group, $P < 0.05$. Values are expressed as experimental/contralateral control muscles, means ± SEM; $n = 6$/group.

Insulin Signaling

We hypothesized that the premature return to baseline in protein synthesis rates may be related to the inability to potentiate the acute effect of feeding. Since insulin signaling is required for the acute response to feeding, we determined IRS-1 levels in the Adult and Old muscles. Consistent with an early return to basal protein synthesis, IRS-1 levels were ∼50–70% lower in Old vs. Adult and were unaffected by exercise (**Figure 5A**). To determine whether the decrease in IRS-1 was a function of age in this rat model, we analyzed quadriceps muscles from 4, 9, 18, 24, and 32-month old F344/BN rats obtained from the NIA tissue repository. Consistent with our Adult and Old groups, levels of IRS-1 in muscle drop more than 10-fold between 4 and 24 months old in the samples from the NIA tissue repository (**Figure 5B**).

Proteasome and Cathepsin L Activity

Proteasome activity was not different from control at 6 h after acute exercise in Adult or Old (**Figure 6**). Eighteen hours after exercise, the activity of the 26S β2, 26S β5, and 20S β5 proteasomal subunits decreased (all $P < 0.05$) in Adult (**Figure 7**). Interestingly, proteasome activity was generally lower in Old vs. Adult (6h = 26S β1, and 20S β2 and β5, main effect of age, all $P < 0.05$; 18 h = 26S β1, β2, β5, and 20S β1, β2, β5, main effect of age, all $P < 0.05$). Cathepsin L activity was greater in Old at the 18 h time point (age effect, $P = 0.008$), but was unchanged by exercise (both age groups, at 6 and 18 h of recovery; **Figure 8**).

DISCUSSION

The present study made age-based comparisons of MPS, ribosome biogenesis, mTORC1 signaling, ER stress, components of insulin signaling and protein degradation after acute eccentric exercise. The major findings are as follows. Precursor ribosomal RNA and total RNA increased similarly between age-groups after resistance exercise. Increases in MPS at 6 and 18 h after exercise were similar between Adult and Old; however, MPS was significantly greater in Adult than Old late in recovery (48 h). mTORC1 signaling was similar in Adult and Old after resistance exercise; however, levels of IRS-1 were significantly lower in Old animals. ER stress was higher- and proteasome activity was lower- at baseline in Old vs. Adult, but neither was induced by exercise.

We previously observed a trend toward increased total RNA 36 h after the same acute resistance exercise protocol used in the present study (West et al., 2016). Here, precursor ribosomal RNA abundance increased at 18 h of recovery and this preceded a significant increase in total RNA at 48 h. These data suggest that exercise-induced enhancement of ribosome biogenesis results in a significant increase in total RNA by 48 h in this model. These data are consistent with the acute stimulation of ribosome biogenesis in humans following resistance exercise (Kim et al., 2007). Consistent with our previous observation (West et al., 2016), the expression of nucleolin and nucleophosmin genes returned to baseline levels by 18 h in the Adult group; conversely, in Old, the expression of these myc target genes remained elevated at 18 h, implying a slower/more prolonged time course

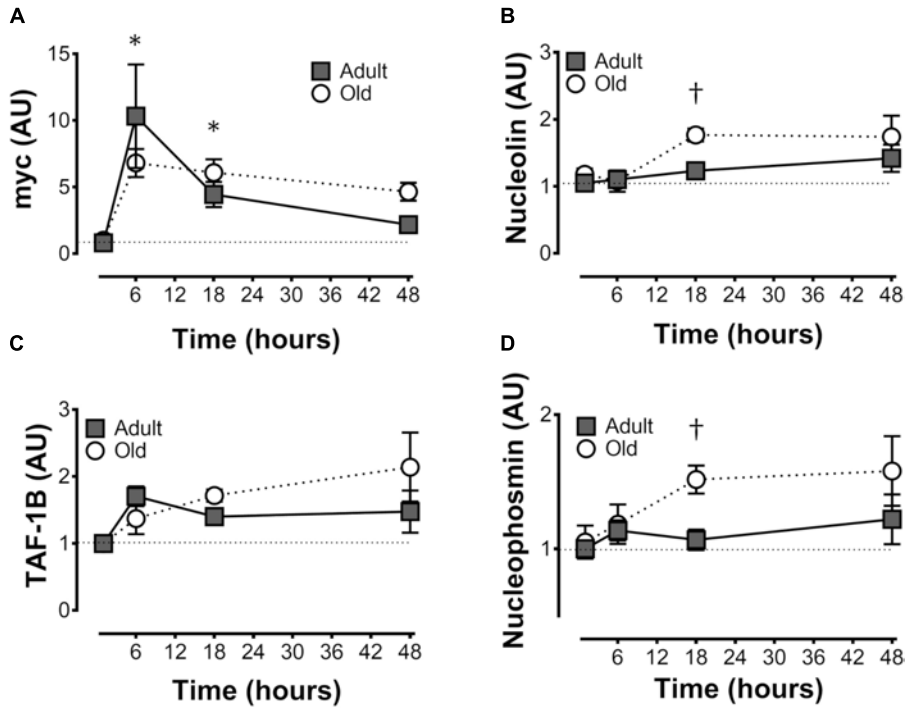

FIGURE 3 | Expression of genes associated with ribosome biogenesis after unilateral resistance exercise in adult (10 months) and old (30 months) rat skeletal muscle. c-Myc **(A)**, nucleolin **(B)**, TATA box binding protein-associated factor RNA Pol I B (TAF1B; **C**), and nucleophosmin **(D)** mRNA abundance. †Difference between Adult and Old at the same time point, $P < 0.05$. Values (means ± SEM) are expressed relative to GAPDH and the contralateral control using the delta-delta Ct method; $n = 6$/group.

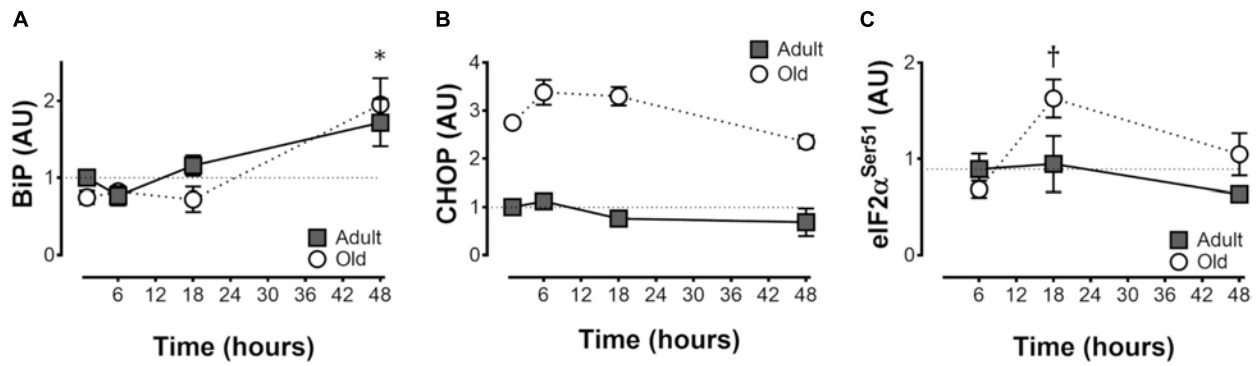

FIGURE 4 | ER stress protein levels after acute unilateral resistance exercise in adult (10 months) and old (30 months) rat skeletal muscle. Endoplasmic reticulum binding protein (BiP; **A**), CCAAT-enhancer-binding protein homologous protein (CHOP; **B**), and phospho eukaryotic initation factor 2α (eIF2α; **C**). *Main effect of time; different from non-stimulated control group, $P < 0.05$. Main effect of age for CHOP, $P < 0.001$ **(B)**. †Difference between Adult and Old at the same time point, $P < 0.05$. $n = 6$/group.

of exercise-mediated induction of these genes. Though the dynamics of the gene responses differed slightly, both Adult and Old were able to increase total RNA at 48 h. Taken together, both adult and old animals appear capable of inducing ribosome biogenesis in response to acute muscle contraction and this similar induction does not explain the age-based differential MPS response.

The finding of apparently normal ribosome biogenesis (at least insofar as rRNA accumulation) after an acute bout of resistance exercise is in contrast to the impaired ribosome biogenesis reported with overload in old mice (Kirby et al., 2015) and resistance training in elderly humans (Stec et al., 2015). One explanation is that old muscle activates ribosome biogenesis in response to an acute exercise stimulus, but that chronic loading results in an impaired response. Future training studies using the present exercise model would be valuable to clarify this hypothesis; in advance, support for this theory comes from work showing that old rats exhibit more metabolic stress

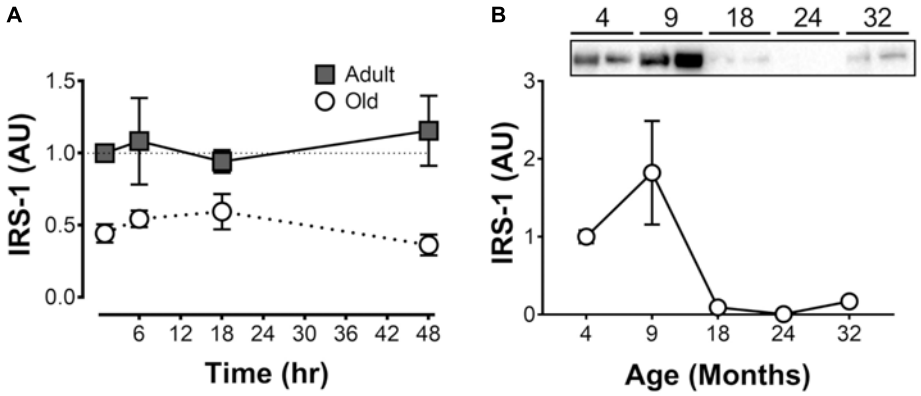

FIGURE 5 | Insulin receptor subunit 1 (IRS-1) protein levels (i) after acute unilateral resistance exercise in adult (10 months) and old (30 months) rat skeletal muscle (**A**; *n* = 6/group), and (ii) across the lifespan **(B)**. Tissue for samples in panel **B** is from the National Institute on Aging Aged Rodent Tissue Bank.

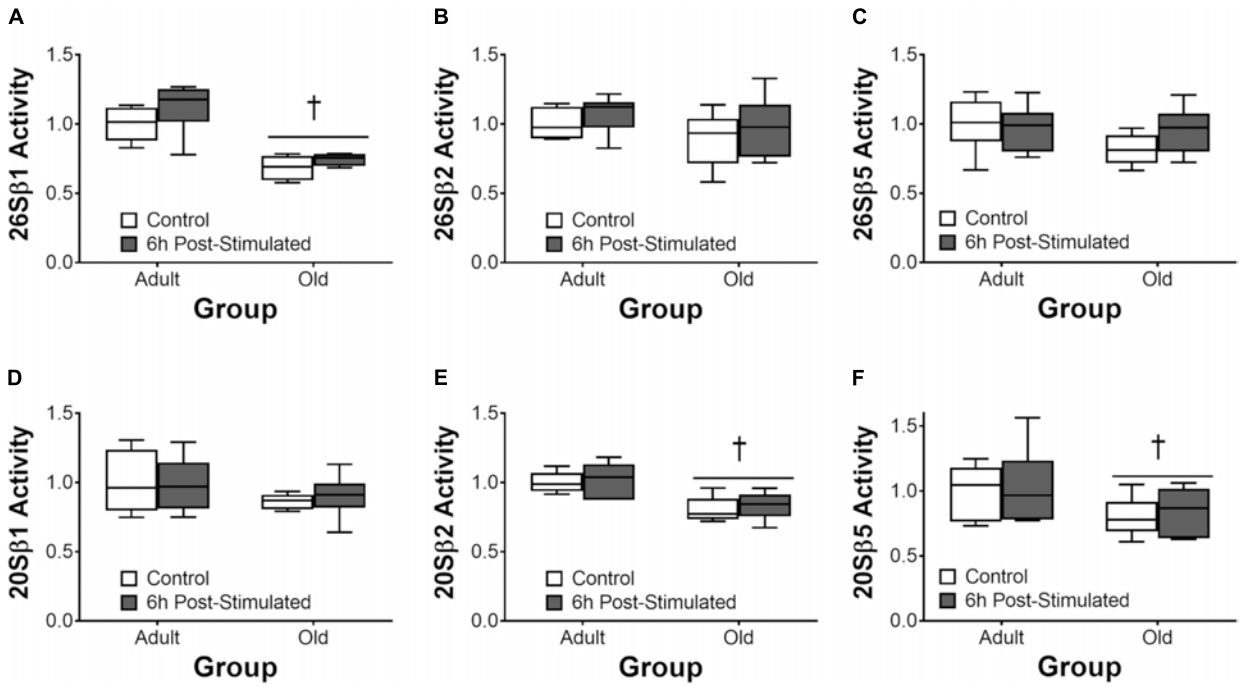

FIGURE 6 | ATP-dependent (26S, **A–C**) and ATP-independent (20S, **D–F**) proteasomal subunit activities 6 h after acute unilateral resistance exercise in adult (10 months) and old (30 months) rat skeletal muscle. Boxes represent 25th to 75th percentiles, horizontal lines within boxes represent medians, and whiskers represent minimums and maximums. *n* = 6/group. †Main effect of age, $P < 0.05$.

with overload and this results in less hypertrophy than young controls (Thomson and Gordon, 2005). Here, our data suggests that aging does not impair the initial accumulation of total RNA, of which ∼85% is ribosomal RNA (Zak et al., 1967), in response to an acute bout of exercise. Ribosome biogenesis occurs predominately in the nucleolus, a process we recently visualized in muscle cells *in vitro* (West et al., 2016). Interestingly, the nucleolus is becoming increasingly recognized as a site for sensing cell stress (Olson, 2004; Nakamura and Kimura, 2017). Thus, future work using ribosome function assays at later time points, and/or in response to repeated stimuli or metabolic stress, would be beneficial to determine whether translational capacity is compromised in old muscle as a result of elevated cellular stress.

The possibility of differences in ribosome assembly/function aside, ribosomal mass accrual appeared to be normal at 48 h post-exercise in Old; in contrast, the protein synthesis response was not; with MPS returning to baseline in Old but not Adult at 48 h. While the specific mechanism for the abbreviated MPS response is unclear, several possibilities exist. First, we have shown that metabolic and ER stress associated with aging (Baehr et al., 2016) can act as a molecular brake on anabolic signaling in skeletal muscle (Hamilton et al., 2014). Whereas the mechanical

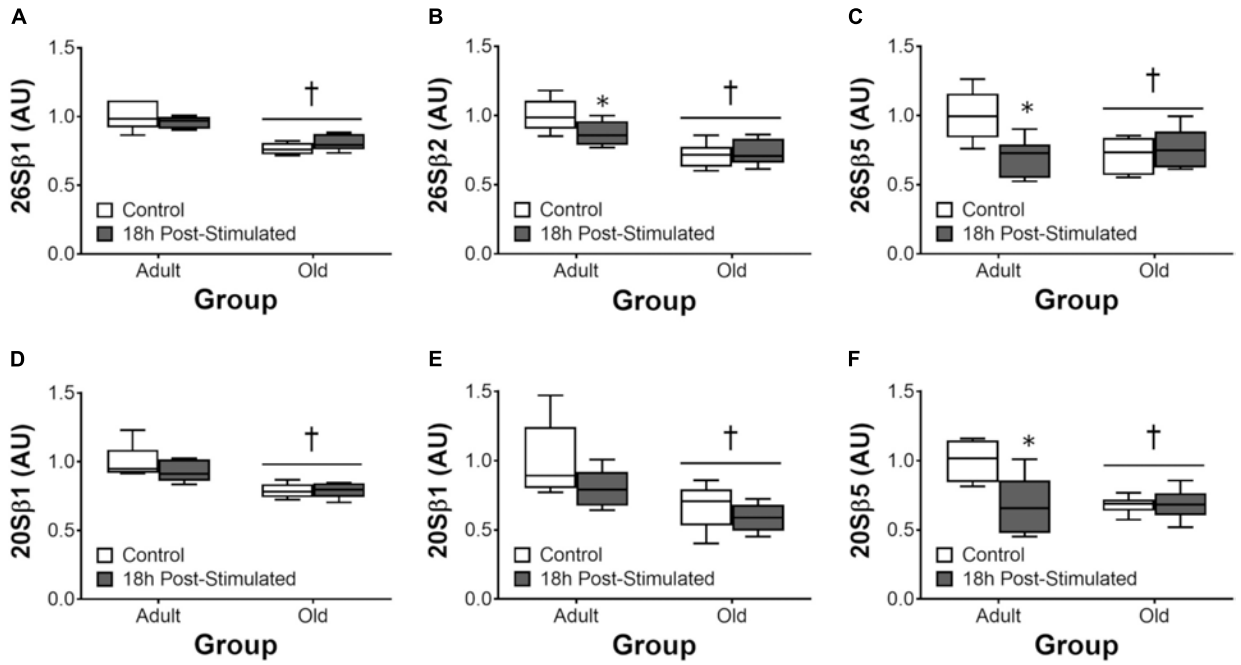

FIGURE 7 | ATP-dependent (26S, **A–C**) and ATP-independent (20S, **D–F**) proteasomal subunit activities 18 h after acute unilateral resistance exercise in adult (10 months) and old rat skeletal muscle. Boxes represent 25th to 75th percentiles, horizontal lines within boxes represent medians, and whiskers represent minimums and maximums. n = 6/group. †Main effect of age, $P < 0.05$. *Difference between exercised and contralateral control muscle within age group.

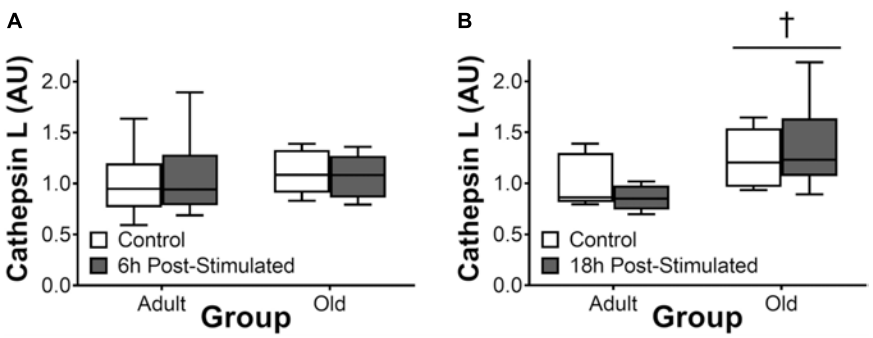

FIGURE 8 | Cathepsin L activity 6 h **(A)** and 18 h **(B)** after acute unilateral resistance exercise in adult (10 months) and old (30 months) rat skeletal muscle. Boxes represent 25th to 75th percentiles, horizontal lines within boxes represent medians, and whiskers represent minimums and maximums. n = 6/group. †Main effect of age, $P < 0.05$.

stimulus to activate mTORC1 signaling did not appear to be intrinsically impaired in Old (S6K, rpS6, and eEF2 were all activated similarly between Adult and Old), it is possible that age-related ER stress may limit protein synthesis. CHOP was higher at all time points in Old, and both BiP and CHOP were elevated at 48 h in Old. Phosphorylated levels of eIF2α were elevated by resistance exercise at 18 h in Old but not Adult. Ser51 phosphorylation of the eIF2 alpha subunit blocks the GEF activity of eIF2B, reducing availability of eIF2-GTP-Met-tRNA$_i$ and inhibiting the global rate of translation initiation (Donnelly et al., 2013; Kashiwagi et al., 2017). Our data showing increased levels of phospho-eIF2α that precede increased BiP, suggests that BiP may not be the trigger to initiate eIF2α phosphorylation as has been proposed (Cui et al., 2011), or at least not in old skeletal muscle in post-exercise recovery. Altogether, the cellular environment in old skeletal muscle, characterized by high CHOP, may down-regulate protein synthesis, which is initially stimulated by intact mTORC1 signaling in Old.

A second potential mechanism to explain the accelerated return to baseline MPS in Old muscle post-exercise is decreased exercise-induced sensitization to feeding (Burd et al., 2011). The early MPS response is driven by the loading stimulus and is independent of insulin/IGF-1 receptor signaling (Spangenburg et al., 2008; Jacobs et al., 2017). This mechanical response appears to be intact in Old since we observed no anabolic resistance in the activation of mTORC1. However, at 48 h, it is possible that the

prolonged increase in MPS is mediated through the potentiation of the feeding response. Unlike the exercise-induced activation of mTORC1 and protein synthesis, the feeding response requires insulin/IGF-1 signaling (Spangenburg et al., 2008; Hamilton et al., 2010; Jacobs et al., 2017). Muscle from both our 30 month old animals and samples from the NIA tissue bank showed that total IRS1 protein content is significantly lower in Old muscle. Since IRS-1 is the first protein recruited to the insulin receptor in response to hormonal activation, it is required for the insulin-dependent activation of PI3-kinase/Akt/mTORC1 and protein synthesis in muscle but not for the mechanical activation of mTORC1 (Hornberger and Chien, 2006). In the NIA tissue bank samples, IRS-1 levels peaked at 9 months and then there was a sharp decline in IRS-1 protein with age. We note that while we have discussed ER stress- and IRS-1-based mechanisms separately above there is evidence to suggest that ER stress inhibits IRS-1 signaling in skeletal muscle as well (Koh et al., 2013). Thus, we speculate that a relative absence of IRS-1 in old muscle – and perhaps reduced activity of remaining IRS-1 – may contribute to a lack of exercise-induced feeding potentiation of MPS at late time points (e.g., 48 h) following resistance exercise.

The feeding-mediated potentiation of human exercise-induced rates of synthesis occurs in the myofibrillar and not the sarcoplasmic protein pool (Burd et al., 2011) and thus, if blunted with aging, may contribute to sarcopenia. Sarcopenia, which is the result of chronically negative net protein balance, is somewhat paradoxical. Our finding of no apparent deficits in translation capacity (on the basis of greater total RNA) add to previous work showing that basal MPS is similar (Volpi et al., 2001) or higher (Smith et al., 2012) in old vs. adult muscles, despite reduced muscle mass (7–17% in the present study; **Table 1**). In non-exercised control muscle, ribosomal density was modestly (+7%) but significantly higher in Old compared with Adult. While there are plausible hypotheses (e.g., increased ribosomal density to compensate for deficits in ribosome function/translational efficiency) – in short, we do not know the mechanism(s) underpinning this observation but note that similar or greater ribosome densities have been reported by others when comparing old vs. young (Haddad and Adams, 2006). Thus, our data indirectly support the notion that sarcopenia is underpinned by recurrent episodes of anabolic resistance.

Our work also supports a mechanism for age-related anabolic dysfunction that is a result of age-related differences in muscle damage. Using the same model, we have previously shown that there is greater membrane damage in old animals in response to exercise due to impaired lateral force transmission (Hughes et al., 2017). Further, supporting evidence shows that old individuals suffer greater contraction-induced muscle injury in response to acute unaccustomed loading (Ploutz-Snyder et al., 2001). The result of this may be that, in the present study, both Adult and Old were able to increase protein synthesis after the exercise bout, but the proteins synthesized in Adult were primarily the myofibrillar proteins involved in muscle growth, whereas in Old more protein synthesis was directed toward injury repair. The surface sensing of translation (SUnSET) method does not characterize which proteins are synthesized following exercise; alternative techniques (e.g., isotopic labeling) that can distinguish which proteins – on a subfraction (Burd et al., 2011) or individual protein (Hines et al., 2015) basis – are being synthesized would enhance our understanding of the acute response to resistance exercise in Adult and Old muscles. From our data, we hypothesize that the impact of aging on myofibrillar protein accretion is two-fold: (1) impaired nutrient signaling decreases the feeding-mediated potentiation of exercise-induced myofibrillar protein synthesis, and (2) relative to adult muscle, protein synthesis in old muscle may be directed toward repairing muscle damage rather than synthesizing the myofibrillar proteins that will increase muscle size and strength.

The UPP is the major protein degradation pathway in skeletal muscle (Rock et al., 1994). Our data showing reduced activity of the UPP in Old at baseline is paradoxical insofar as the net protein balance equation and is in contrast to previous reports (Hepple et al., 2008; Altun et al., 2010). Nevertheless, our data is consistent with the findings of others (Anvar et al., 2011; Hwee et al., 2014), leading us to hypothesize that age-related reductions – not elevations – in skeletal muscle proteasome activity contribute to cellular dysfunction which may in turn contribute to the gradual pathological phenotype characterized by sarcopenia, as has been suggested to occur with diabetes (Al-Khalili et al., 2014). The decrease in proteasome function that we observed in Old may have contributed to the higher ER stress levels (via reduced ER-associated degradation) we observe in old skeletal muscle, here and previously (Baehr et al., 2016). In the context of exercise, the present findings are in agreement with our previous observation (West et al., 2016) of no induction of proteasome activity following acute resistance exercise. Likewise, cathepsin L activity was not significantly altered by resistance exercise, and was either no different (6 h post-exercise) or elevated (18 h) in Old vs. Adult overall. Thus, collectively, we do not detect significant exercise-induced increases in protein degradation by the proteasome or autophagy systems, but suspect that age-related impairments in the basal activity of these pathways contribute to age-related impairments in myofiber remodeling and adaptation to loading.

Limitations

We acknowledge that the animal model used presents several limitations insofar as the generalizability of our findings to the geriatric population. First, the tibialis anterior muscle that was examined is comprised of a high proportion of fast-twitch fibers, higher than human ambulatory muscles. Accordingly, given the impact of fiber type on protein metabolism (Dickinson et al., 2010; Goodman et al., 2012.), it is unclear whether the same findings would be observed in the more oxidative/"mixed" fiber-types seen in people. Examining the impact of aging on outcomes presented herein in muscles of a different fiber type composition (e.g., soleus) would be an interesting avenue for future work. Second, we intentionally used a model of maximal motor unit activation to address the issue of whether or not age-related differences in motor unit activation (Kamen et al., 1995) contribute to age-related anabolic resistance to resistance exercise. This was a mechanisms-targeted strategy to remove motor unit activity as a variable and we acknowledge that this

exercise paradigm does not mimic the exercise patterns of the geriatric population. Finally, because the electrically stimulated eccentric contractions used in the present study do not mimic the general exercise patterns of geriatric populations, our findings should not be applied indiscriminately to any such population undertaking resistance exercise. Having said that, researchers contend that electrical stimulation is an effective strategy to attenuate muscle loss in elderly and clinical populations (Wall et al., 2012), and that eccentric exercise is safe, feasible, and relevant even in clinical populations (LaStayo et al., 2014). Further, work showing that when lifting a weight to concentric failure humans recruit all of the motor units within the active muscle (Burd et al., 2012a), suggesting that older individuals can benefit from these results by lifting a weight to failure. Thus, while our research model does not represent the general exercise patterns of elderly, the model remains within the bounds of a physiologically relevant loading stimulus while yielding mechanistic insight into the response to exercise in young vs. old individuals.

CONCLUSION

In conclusion, our data suggest that when motor units are fully recruited to undergo maximal eccentric loading, the capacity of old muscle to elevate MPS in response to an acute bout of exercise is initially intact but the duration of the response is truncated. This response does not appear to be related to age-related differences in ribosome biogenesis, since ribosomal mass accumulates similarly in Adult and Old muscle at 48 h. Rather injury, ER stress and/or impaired exercise-induced feeding potentiation are more likely to contribute to age-related anabolic resistance in late post-exercise recovery. We observe no induction of proteasome activity following acute resistance exercise and hypothesize that age-related reductions – not elevations – in skeletal muscle proteasome activity contribute to cellular dysfunction which may in turn contribute to sarcopenia.

AUTHOR CONTRIBUTIONS

DW, SB, and KB designed the study. DW and KB drafted the manuscript. All authors collected and analyzed the data, responsible for revising the intellectual content of the manuscript, and reading and approving the final version of the manuscript.

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Aging and Strength Training Influence Knee Extensor Intermuscular Coherence During Low- and High-Force Isometric Contractions

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Aging is associated with reduced maximum force production and force steadiness during low-force tasks, but both can be improved by training. Intermuscular coherence measures coupling between two peripheral surface electromyography (EMG) signals in the frequency domain. It is thought to represent the presence of common input to alpha-motoneurons, but the functional meaning of intermuscular coherence, particularly regarding aging and training, remain unclear. This study investigated knee extensor intermuscular coherence in previously sedentary young (18–30 years) and older (67–73 years) subjects before and after a 14-week strength training intervention. YOUNG and OLDER groups performed maximum unilateral isometric knee extensions [100% maximum voluntary contraction (MVC)], as well as force steadiness tests at 20 and 70% MVC, pre- and post-training. Intermuscular (i.e., EMG-EMG) coherence analyses were performed for all (three) contraction intensities in vastus lateralis and medialis muscles. Pre-training coefficient of force variation (i.e., force steadiness) and MVC (i.e., maximum torque) were similar between groups. Both groups improved MVC through training, but YOUNG improved more than OLDER (42 ± 27 Nm versus 18 ± 16 Nm, $P = 0.022$). Force steadiness did not change during 20% MVC trials in either group, but YOUNG demonstrated increased coefficient of force variation during 70% MVC trials (1.28 ± 0.46 to 1.57 ± 0.70, $P = 0.01$). YOUNG demonstrated greater pre-training coherence during 20% and 70% MVC trials, particularly within the 8–14 Hz (e.g., 20%: 0.105 ± 0.119 versus 0.016 ± 0.009, $P = 0.001$) and 16–30 Hz (20%: 0.063 ± 0.078 versus 0.012 ± 0.007, $P = 0.002$) bands, but not during 100% MVC trials. Strength training led to increases in intermuscular coherence within the 40–60 Hz band during 70% MVC trials in YOUNG only, while OLDER decreased within the 8–14 Hz

band during 100% MVC trials. Age-related differences in intermuscular coherence were observed between young and older individuals, even when neuromuscular performance levels were similar. The functional significance of intermuscular coherence remains unclear, since coherence within different frequency bands did not explain any of the variance in the regression models for maximum strength or force steadiness during 20 and 70% MVC trials.

Keywords: alpha-motoneuron, motor control, voluntary contraction, lower-limb, Piper rhythm, beta-band

INTRODUCTION

Aging is associated with degenerations in neural functioning that reduce performance during voluntary force production tasks. For example, force steadiness during low- and moderate-force isometric contractions is poorer in older individuals (Tracy and Enoka, 2006; Griffin et al., 2009) and particularly so in those with a history of falls (Carville et al., 2007). This is accompanied by less (larger) motor neurons/units (Lexell et al., 1988), which increases the size of each motor unit (Piasecki et al., 2016), and by greater variability in motor unit discharge rates (Tracy et al., 2005; Kallio et al., 2012). Strength training, which is typically used to combat the deleterious effects of aging on strength and muscle mass, improves activation of motor units during high-force tasks in both young and older individuals (Häkkinen and Häkkinen, 1995; Van Cutsem et al., 1998; Kamen and Knight, 2004; Walker et al., 2014). However, the processes within the neural circuitry that reduce variability in motor unit discharge rates and generate increased force output remain unknown.

One approach in neurophysiology is to compute coherence between two biological signals. Coherence is a measure of correlation between two signals in the frequency domain (Halliday et al., 1995). Coherence analysis estimates the amount of common neural input between two sites during voluntary motor tasks (Ushiyama and Ushiba, 2013). Corticomuscular coherence has been assessed between signals representing cortical (by electroencephalography or magnetoencephalography) and muscular [by electromyography (EMG)] activities. Alternatively, intermuscular (EMG–EMG) coherence between two muscles' activities has been assessed. Broadly, intermuscular coherence may reflect shared neural inputs from cortical, subcortical and spinal influences (Grosse et al., 2002). Although not explicitly known, coherence within the 8–14 Hz frequency band may be related to Ia afferent feedback (Lippold, 1970), although somatosensory feedback to the cortex appears to operate at <3 Hz (Bourguignon et al., 2017). Coherence within the 15–30 Hz frequency band suggests common corticospinal input (Fisher et al., 2012) potentially driven by sensorimotor cortex oscillations (Bourguignon et al., 2017), while 40–60 Hz frequency band has potentially reticulospinal origin (Garcia-Rill et al., 2016). Intermuscular coherence is relatively easy to measure and compute for large populations (Jaiser et al., 2016) and may have clinical relevance (Fisher et al., 2012; Larsen et al., 2017). Furthermore, intermuscular coherence methods allow examination of high-force contractions (>70% of maximum), whereas EEG signals may be contaminated by muscular artifacts when examining high-force corticomuscular coherence.

Since corticomuscular coherence is most robust within 15–30 Hz band during steady low-force isometric contraction (Conway et al., 1995; Baker et al., 1997; Salenius et al., 1997), scientific investigation has predominantly focused on these frequencies. Coherence at 15–30 Hz has been suggested to be related to a neural strategy for precision tasks, reflecting a contribution from the corticospinal tract (Fisher et al., 2012). Concurrently, it has long been known that voluntary contractions can exhibit oscillations at higher frequencies, e.g., the so-called Piper rhythm at 40–60 Hz (Piper, 1907). Corticomuscular coherence appears to be force-dependent, since higher force contractions demonstrate greater Piper coherence (Brown et al., 1998; Mima et al., 1999). Summarizing several studies' findings, it appears that contraction intensities above 60% of maximum voluntary contraction (MVC) are required to observe this force-related increase in 40–60 Hz coherence, although this is not present in all muscles (Ushiyama et al., 2012). Since 15–30 Hz corticomuscular coherence has been shown to be lower in trained individuals (weightlifters and ballet dancers) compared to untrained individuals (Ushiyama et al., 2010), it would be of interest to determine whether strength training influences 40–60 Hz coherence in a contrasting fashion.

However, the intermuscular coherence method has come under scrutiny as to its ability to determine the amount of common neural input due to the potentially decorrelating influence of multiple sources of common synaptic input (Farina et al., 2014). Hence, it is of interest to determine whether intermuscular coherence can distinguish between groups and possible intervention effects if this method is to gain more widespread usage. Consequently, the present study aimed to determine; (1) the magnitude of intermuscular coherence during low- and high-force isometric knee extensions in healthy young and older adults and (2) possible short-term strength training-induced changes in intermuscular coherence during low- and high-force contractions in young and older adults. In order to estimate potential functional significance of coherence, regression models were constructed to test whether intermuscular coherence strengths within 2–6, 8–14, 16–30, and 40–60 Hz frequency bands predicted force steadiness and/or maximum force production during the isometric contractions. Regression models were assessed for; (1) both pre-training force steadiness and maximum force values and (2) the changes in coherence and force steadiness/maximum force due to strength training.

MATERIALS AND METHODS

Subjects

Sixteen young (YOUNG, age range: 18–31, 4 men and 12 women) and 16 older (OLDER, age range: 66–73, 8 men and 8 women) individuals volunteered for the study. None of the subjects had any history of neurological conditions including diabetes mellitus, none took any neurotropic medication, and none had any lower limb injuries or disabilities. Additionally, all older subjects underwent a medical examination to ensure there were no contraindications to performing maximal effort testing and strength training.

One young and three older subjects did not complete the strength training intervention for various reasons and dropped-out of the study (illness not related to the study, loss of interest, one older man suffered injury during the training). Significant intermuscular coherence across a broad frequency spectrum was observed in 13 out of 15 YOUNG and 11 out of 13 OLDER that completed 14 weeks of strength training. These subjects were then taken forward into subsequent analyses. The final characteristics of the subjects in both age groups were as follows: YOUNG: age 25 ± 4 years, height 170 ± 10 cm, body mass 64 ± 11 kg, body mass index 22 ± 4 (3 men and 10 women), OLDER: age 69 ± 3 years, height 166 ± 9 cm, body mass 72 ± 12 kg, body mass index 26 ± 3 (5 men and 6 women).

All subjects provided written informed consent prior to the study. The study was approved by the ethical committee of the University of Jyväskylä and was performed according to the Declaration of Helsinki.

Test Procedures

Prior to testing, the subjects attended a familiarization visit to the lab. The purpose of this session was to individualize the settings of the custom-built isometric dynamometer, teach the correct contraction techniques for maximal and submaximal unilateral isometric knee extension tasks, allow practice trials, and place indelible ink tattoos marking the EMG locations of m. vastus lateralis (VL) and vastus medialis (VM). Subjects performed unilateral knee extension trials with the right leg and a knee angle of 110°. Non-elastic straps about the ankle, knee and hip secured the subjects to the dynamometer and prevented movement during contraction. The subjects' arms were folded across their chest. They were instructed to be as relaxed as possible in the upper body, and to concentrate on producing force through only extension about the knee.

One week later, subjects reported to the lab for their pre-training tests. Following preparation and placement of EMG electrodes, subjects performed four maximum voluntary contractions (MVC). Subjects were instructed to perform each trial as "hard and as fast as possible" and were given constant verbal encouragement. Real-time visual feedback of force production was provided during all tasks. The best trial (i.e., highest force) was taken as that individual's MVC performance. Thereafter, force levels equivalent to 20 and 70% of MVC were calculated and displayed as horizontal bars on the screen. Subjects then performed 4 × 30 s contractions at approximately 20% MVC with 60 s rest between trials. Next, subjects performed four sets of 4 × 6 s contractions at approximately 70% MVC with 15 s rest between trials and 90 s rest between sets. During the submaximal trials, subjects were instructed to maintain a constant force level as close as possible to the target (horizontal) bar. Force steadiness was taken as the averaged Coefficient of Variation in force (CV force %) of all trials (CV = SD/mean*100). Finally, the subjects performed 1–2 MVC trials at the end of the testing session to ensure that no fatigue had occurred due to the test procedures (mean change in force from the beginning to the end of the testing was 575 ± 108N to 565 ± 88N, $P = 0.332$).

Following these measurements, subjects completed 14 weeks of supervised and progressive strength training in the University gym (details are given below) prior to returning for post-training tests (**Figure 1**). These tests took place 7 days after the last training session to eliminate any residual fatigue from training, and the test time-of-day was matched to that of the pre-training test (±1 h).

During each maximal and submaximal knee extension trial a square-pulse trigger was manually generated to the recording software once the subjects had reached a steady force level. This pulse was used to synchronize the start of the analysis (see later). Upon completion of the contraction time (30 s for 20%, 6 s for 70%, and 3 s for 100% MVC trials), a second square-pulse trigger was manually generated to synchronize the end of the trial/analyses, and then the subject was instructed to relax (**Figure 2**).

Electromyography Recordings

Following shaving and skin preparation, bipolar Ag/AgCl electrodes (5 mm diameter, 20 mm inter-electrode distance) were positioned on VL and VM of the right leg according to SENIAM guidelines. Placement was in-line with the orientation of the underlying fascicles, and guided by the tattoos to ensure reproducibility from session to session. Raw EMG signals were amplified at a gain of 500 (bandwidth 10–500 Hz, common mode rejection ratio >100 dB, input impedance >100 MΩ, baseline noise <1 μV rms) and sampled at 2000 Hz. Raw signals were sent from a hip-mounted pack to a receiving box (Telemyo 2400R, Noraxon, Scottsdale, United States), then were relayed to an analog-to-digital converter (Micro1401, Cambridge Electronic Design, United Kingdom) and recorded by Signal 4.10 software (Cambridge Electronic Design, United Kingdom).

Intermuscular Coherence Analyses

Coherence analyses were performed using Matlab (Mathworks, Natick, MA, United States) using customized scripts in a similar manner as previously reported (Jaiser et al., 2016). Raw data was visually inspected, and in the rare case that single trials were performed incorrectly or demonstrated signal disruption or excessive noise, they were removed from the analyses. Analysis used the time window between the start and end triggers described above (∼30 s for each of the four 20% MVC trials, ∼6 s for each of the sixteen 70% MVC trials, and ∼3 s for the four MVC trials). Trials of the same task were collated to yield total recording lengths of ∼120, 96, and 12 s for the 20,

FIGURE 1 | Isometric knee extension experimental setup.

70, and 100% MVC tasks, respectively. EMG signals were full-wave rectified prior to analyses. Available data was separated into non-overlapping windows 1024 samples in length, which were subjected to fast Fourier transform. This gave a frequency resolution of 1.95 Hz.

Denoting the Fourier transform of the two signals in the l'th window at frequency λ as $F_{1,l}(\lambda)$ and $F_{2,l}(\lambda)$, the auto-spectrum of the first signal was given by:

$$f_{11}(\lambda) = \frac{1}{L}\sum_{l=1}^{L} \mathrm{F}_1, l(\lambda) F_1, l(\lambda)^*$$

The cross-spectrum was calculated as:

$$f_{12}(\lambda) = \frac{1}{L}\sum_{l=1}^{L} \mathrm{F}_1, l(\lambda) F_2, l(\lambda)^*$$

Here * denotes the complex conjugate, and L is the total number of sections. The pre-training L-values were as follows; intermuscular coherence 20% = 226 ± 2, 70% = 179 ± 4, 100% = 28 ± 2 (post-training values were similar).

Coherence was calculated as the cross-spectrum normalized by the auto-spectrum:

$$\mathrm{C}(\lambda) = \frac{\left|f_{12}(\lambda)\right|^2}{f_{11}(\lambda) f_{22}(\lambda)}$$

coherence above Z was considered significantly above chance, according to the formula developed by Brillinger (1981) and given by Rosenberg et al. (1989):

$$Z = 1 - \alpha^{1/(L-1)}$$

where the significance level α was set to 0.05.

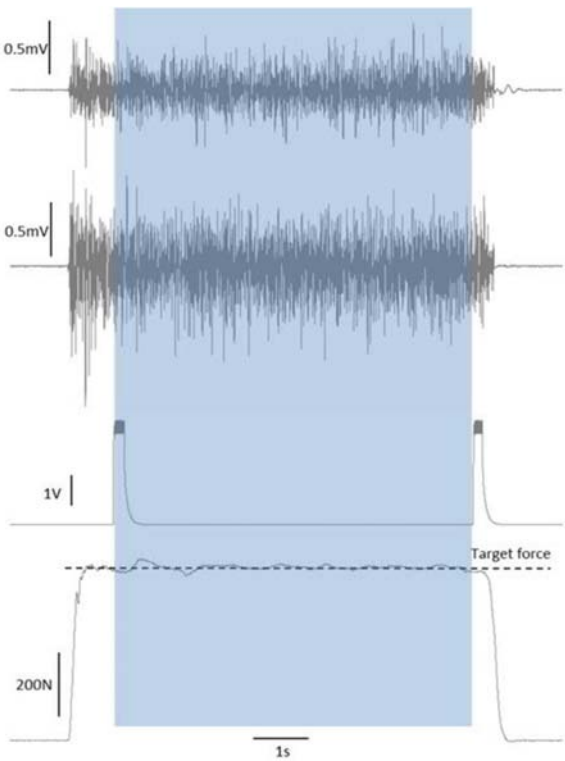

FIGURE 2 | Synchronization of force and EMG signals by start/end of trial square-pulse triggers. The shaded area shows the single-trial duration that was taken forward into the analyses.

Intermuscular coherence spectra for 0–100 Hz were averaged across subjects in each age group (i.e., YOUNG and OLDER). The group significance level was then determined using the method of

Evans and Baker (2003). Thereafter, coherence was sectioned into 2–6, 8–14, 16–30, and 40–60 Hz bands and the average coherence within each window used to compare between groups and time points.

Body Composition Measurements

On a separate occasion, participants visited the lab after a 12-h overnight fast. After determination of height by a fixed wall-mounted scale, participants underwent full body scanning by dual-energy X-ray absorptiometry (DXA) in minimal clothing (LUNAR Prodigy Advance with encore software version 9.3, GE Medical Systems, United States). The legs were separated by a polystyrene block and secured by inelastic straps about the ankles to ensure no movement during the scanning and accurate replacement after the training period. An operator-defined Range-of-Interest for the thigh region was manually produced from anatomical landmarks; the same operator performed all analyses. The proximal and distal ends of the Range-of-Interest were the apex of the greater trochanter and knee joint space, respectively. Thigh region fat mass and fat-free mass was determined from the scans to assess morphological adaptations to strength training.

Strength Training

Subjects reported to the University gym twice per week on non-consecutive days – typically Monday/Thursday or Tuesday/Friday. Each training session consisted of around 9 exercises for all muscle groups (i.e., whole body training), however, all leg exercises were performed first since this was the primary target muscle group. The specific training program is given in **Table 1**. Loads progressed incrementally throughout the training period and the subjects were encouraged to lift the load until momentary failure [i.e., repetition maximum (RM)]. In the present study, subjects performed sets of 16 RM in the beginning of training progressing to sets of 6 RM at the end of the training period. Lighter load sets were included toward the end of the training period, in which the concentric phase was performed with maximum velocity but momentary failure was not realized. In general, this type of strength training program can be considered to be linearly periodized, progressing from muscular endurance-focused to hypertrophy- and maximum strength-focused training, and finishing with power-focused training. Subjects were instructed how to perform each exercise and technique was constantly monitored by qualified instructors. The tempo for muscular endurance and hypertrophy trainings was 2 s for concentric and 2 s for eccentric phases, while tempo for maximum strength and power trainings was as fast as possible for concentric and either as fast as possible or 2 s for eccentric phases depending on the type of exercise (noted in **Table 1**). Subjects were allowed to continue their habitual physical activities, such as low intensity walking, cycling, and swimming at a frequency of 1–3 times per week, during the study period.

Statistical Methods

Results are reported as means and standard deviations (SD). To compare coherence-frequency curves both within- and between-groups, *Z*-scores via a hyperbolic arctan transform were generated according to the methods of Jaiser et al. (2016). Individual subject's data were inspected and only subjects showing significant broad-spectra coherence were included in this study. Statistically significant differences for each frequency bin, based on the *Z*-score analysis, was estimated using Monte-Carlo simulations and are highlighted in **Figures 4** and **6**.

Thereafter, coherence within each frequency band was also assessed statistically within- and between-groups using SPSS software version 24 (IBM, New York, NY, United States). Similar results were obtained using peak and averaged coherence in each of the frequency bands. For clarity, only average coherence results are reported here. Average coherence values across the various frequency bands were not normally distributed and so the coherence data were log 10 transformed to allow parametric tests to be performed. Repeated measures ANOVA (2 time × 2 group) was used on maximum strength and force steadiness, as well as average coherence across the four frequency bands. *Post hoc* tests were performed with Bonferroni adjustments.

Linear multiple regression analysis was performed using the following predictors; average intermuscular coherence in 2–6, 8–14, 16–30, and 40–60 Hz frequency bands, fat-free mass and fat mass to predict force steadiness and/or maximum force production using the stepwise method in SPSS.

RESULTS

Maximum Strength and Force Steadiness Pre- and Post-training

Pre-training 100% MVC torque (YOUNG: 195 ± 53 Nm, OLDER: 178 ± 53 Nm) did not differ between groups. Similarly, pre-training force steadiness did not differ between the age groups during either 20% MVC trials (YOUNG: 0.35 ± 0.15%, OLDER: 0.28 ± 0.09%) or 70% MVC trials (YOUNG: 1.28 ± 0.46%, OLDER: 0.96 ± 0.34%).

Significant main effects for time ($F = 38.2$, $P < 0.001$) and time × group ($F = 6.1$, $P = 0.022$) were observed in MVC. Strength training led to an increase in MVC in both groups (YOUNG: 195 ± 53 Nm to 236 ± 54 Nm, $P = 0.001$; OLDER: 178 ± 53 Nm to 196 ± 49 Nm, $P = 0.005$). However, the improvements obtained by YOUNG were significantly greater than those obtained by OLDER (42 ± 27 Nm versus 18 ± 16 Nm, $P = 0.022$).

No main effects for force steadiness during 20% MVC trials were observed (YOUNG: 0.35 ± 0.15% and 0.37 ± 0.20%; OLDER: 0.28 ± 0.09% and 0.26 ± 0.07%). However, significant main effects for time ($F = 5.6$, $P = 0.027$), time × group ($F = 5.8$, $P = 0.025$) and group ($F = 5.8$, $P = 0.025$) were observed in force steadiness during 70% MVC trials. YOUNG demonstrated significant worsening of force steadiness during 70% MVC trials (1.28 ± 0.46% to 1.57 ± 0.70%, $P = 0.01$), and the change was statistically different compared to that in OLDER (0.29 ± 0.34% versus −0.02 ± 0.2%, $P = 0.025$). Changes in maximum force production and force steadiness are shown in **Figure 3**.

TABLE 1 | Progressive strength training program completed by all subjects.

Weeks	Main training goal	Session	Exercise	Sets	Reps	%1-RM	Tempo	Inter-set rest
1–2	Muscular endurance	1	Leg press	3	14–16	40–60%	2:2	60 s
			Knee extension	2	14–16	40–60%		
			Knee flexion	2	14-16	40–60%		
			Chest press	2	14–16	40–60%		
			Lat pulldown	2	14–16	40–60%		
			Triceps extension	2	14–16	40–60%		
			Ab curl	2	16–20	BM		
			Back extension	2	16–20	BM		
		2	Leg press	3	14–16	40–60%	2:2	60 s
			Knee extension	2	14–16	40–60%		
			Knee flexion	2	14–16	40–60%		
			Shoulder press	2	14–16	40–60%		
			Seated row	2	14–16	40–60%		
			Biceps curl	2	14–16	40–60%		
			Seated calf-raise	2	14–16	40–60%		
			Ab curl	2	16–20	40–60%		
			Back extension	2	16–20	40–60%		
3–5	Hypertrophy	1	Leg press	3	10–12	70–80%	2:2	60 s
			Knee extension	3	10–12	70–80%		
			Knee flexion	2	10–12	70–80%		
			Chest press	3	10–12	70–80%		
			Lat pulldown	2	10–12	70–80%		
			Triceps extension	2	10–12	70–80%		
			Standing calf-raise	3	10–12	70–80%		
			Ab curl	2	14–16	50–70%		
			Back extension	2	14–16	50–70%		
		2	Leg press	3	10–12	70–80%	2:2	60 s
			Knee extension	2	10–12	70–80%		
			Knee flexion	3	10–12	70–80%		
			Shoulder press	2	10–12	70–80%		
			Seated row	3	10–12	70–80%		
			Biceps curl	2	10–12	70–80%		
			Seated calf-raise	3	10–12	70–80%		
			Ab curl	2	14–16	50–70%		
			Back extension	2	14–16	50–70%		
6–7	Hypertrophy	1	Leg press	4	8–10	80–85%	2:2	60 s
			Knee extension	3	8–10	80–85%		
			Knee flexion	2	8–10	80–85%		
			Chest press	4	8–10	80–85%		
			Lat pulldown	2	8–10	80–85%		
			Triceps extension	2	8–10	80–85%		
			Standing calf-raise	3	8–10	80–85%		
			Ab curl	2	10–12	70–80%		
			Back extension	2	10–12	70–80%		
		2	Leg press	4	8–10	80–85%	2:2	60 s
			Knee extension	2	8–10	80–85%		
			Knee flexion	3	8–10	80–85%		
			Shoulder press	2	8–10	80–85%		
			Seated row	4	8–10	80–85%		

(Continued)

TABLE 1 | Continued

Weeks	Main training goal	Session	Exercise	Sets	Reps	%1-RM	Tempo	Inter-set rest
			Biceps curl	2	8–10	80–85%		
			Seated calf-raise	3	8–10	80–85%		
			Twisting Ab	2	10–12	70–80%		
			Back extension	2	10–12	70–80%		
8–9	Maximum strength	1	Leg press	3	5–8	85–90%	2:2	120 s
			Knee extension	3	6–8	85–90%		
			Knee flexion	3	6–8	85–90%		
			Lunge*	2	12–14	60–70%		
			Standing calf-raise	2	12–14	60–70%		
			Seated calf-raise	2	8–10	80–85%		
			Bench press*	2	12–14	60–70%		
			Db shoulder press*	2	12–14	60–70%		
			Ab crunch	2	12–14	BM		
			Back extension	2	12–14	BM		
		2	Smith-machine squat	3	5–8	85–90%	2:2	120 s
			Knee extension	3	6–8	85–90%		
			Knee flexion	3	6–8	85–90%		
			Db deadlift*	2	12–14	60–70%		
			Standing calf-raise	4	12–14	60–70%		
			Bent-over row*	2	12–14	80–85%		
			Assisted pull-up	2	12–14	60–70%		
			Ab crunch	2	12–14	BM		
			Back extension	2	12–14	BM		
10–12	Maximum strength and power	1	Leg press	3	4–6	90–95%	1:2	120 s
			Knee extension	3	6–8	85–90%	1:2	
			Knee flexion	3	6–8	85–90%	1:2	
			Standing calf-raise	3	7–8	85–90%	1:2	
			Calf jumps*	1	7–8	BM	0:0	
			Lunge jumps*	2	5	BM	0:2	
			Bench press*	3	10–12	70–80%	2:2	
			Db shoulder press*	2	10–12	70–80%	2:2	
			Ab crunch	2	14–16	BM	2:2	
			Back extension	2	14–16	BM	2:2	
		2	Smith-machine squat	3	4–6	90–95%	1:2	120 s
			Knee extension	3	6–8	85–90%	1:2	
			Knee flexion	3	6–8	85–90%	1:2	
			Standing calf-raise	3	7–8	85-90%	1:2	
			Calf jumps*	1	7–8	BM	0:0	
			CMJ*	2	5	BM	0:2	
			Bent-over row*	3	10–12	70–80%	2:2	
			Assisted pull-up	2	10–12	70–80%	2:2	
			Ab crunch	2	14–16	BM	2:2	
			Back extension	2	14–16	BM	2:2	
13–14	Power	1	Leg press	3	4–6	50–60%	0:2	120 s
			Knee extension	3	4–6	90–95%	1:2	
			Knee flexion	3	4–6	90–95%	1:2	
			Standing calf-raise	1	7–8	85–90%	1:2	
			Calf jumps*	3	7–8	BM	0:0	
			Lunge jumps*	3	5	BM	0:2	
			Pec Deck	3	8–10	80–85%	2:2	

(Continued)

TABLE 1 | Continued

Weeks	Main training goal	Session	Exercise	Sets	Reps	%1-RM	Tempo	Inter-set rest
			Assisted dips*	3	8–10	80–85%	2:2	
			Ab crunch	2	16–20	BM	2:2	
			Back extension	2	16–20	BM	2:2	
		2	Smith-machine squat	3	4–6	50–60%	0:2	120 s
			Knee extension	3	4–6	90–95%	1:2	
			Knee flexion	3	4–6	90–95%	1:2	
			Standing calf-raise	1	7–8	85–90%	1:2	
			Calf jumps*	3	7–8	BM	0:0	
			CMJ*	3	5	BM	0:2	
			Seated row	3	8–10	80–85%	2:2	
			Upright row*	3	8–10	80–85%	2:2	
			Ab crunch	2	16–20	BM	2:2	
			Back extension	2	16–20	BM	2:2	

*Tempo refers to the time of the concentric:eccentric phase of the lift in seconds. 1-RM, one-repetition maximum; Ab, abdominal; Db, dumbbell; BM, body mass; CMJ, countermovement jump; *, free-weight exercise.*

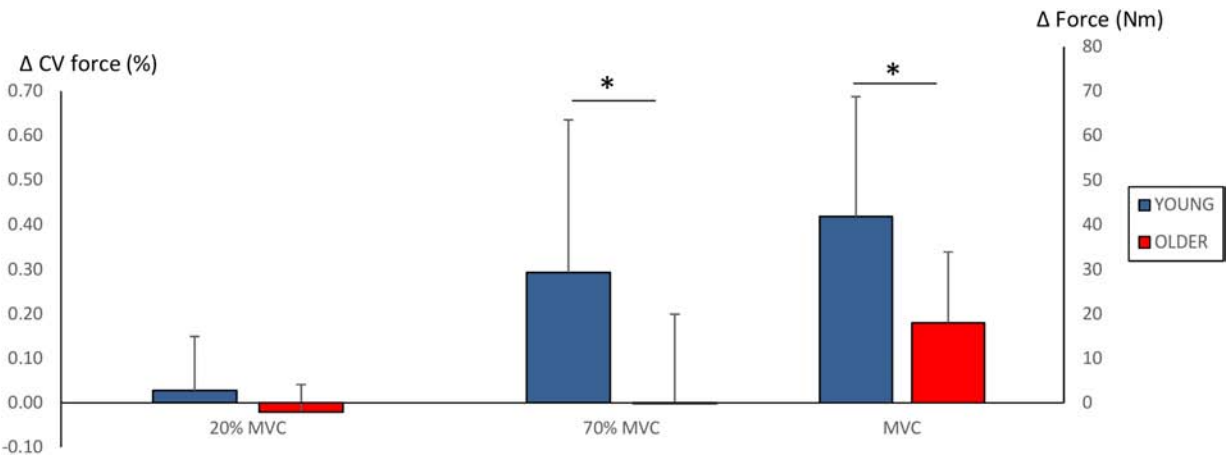

FIGURE 3 | Changes (Δ, mean ± SD) in force steadiness (CV of force %) during 20 and 70% MVC trials and maximum force production (Nm) during 100% MVC trials. *$P < 0.0125$ between-groups comparisons. Note that increased CV of force % (i.e., greater force fluctuation) reflects poorer force steadiness.

Effect of Aging on Intermuscular Coherence

Figure 4 shows pre-training intermuscular coherence over 0–100 Hz during 20, 70, and 100% MVC trials. Both groups showed relatively large coherence levels over approx. 0–60 Hz, with the exception of OLDER during 20% MVC trials. Significant coherence was a robust finding across a majority of subjects (**Figures 4B,D,F**). Using *Z*-score comparisons, YOUNG demonstrated significantly larger intermuscular coherence compared to OLDER over frequencies of approximately 8–36 Hz during both 20 and 70% MVC trials ($P < 0.05$, **Figures 4A,C**). There were no differences between age-groups during 100% MVC trials (**Figure 4E**).

Pre-training average coherence within the frequency bands 2–6, 8–14, 16–30, and 40–60 Hz is shown in **Figure 5**. Significant main effects for group were observed in 8–14, 16–30, and 40–60 Hz coherence during 20% MVC trials ($F = 5.2–14.6$, $P = 0.032–0.001$) and in 8–14 and 16–30 Hz coherence during 70% MVC trials ($F = 11.5$, $P = 0.003$). YOUNG demonstrated significantly greater coherence levels than OLDER in the 8–14 and 16–30 Hz bands during 20 and 70% MVC trials pre- (**Figures 5A,B**) and post-training (not shown).

Effect of Strength Training on Intermuscular Coherence

Few changes were observed due to strength training in intermuscular coherence over 0–100 Hz during 20 and 70% MVC trials (**Figure 6**). During 20% MVC trials, YOUNG had significantly reduced coherence at 18–20 Hz post-training ($P < 0.05$, **Figure 6A**). Whereas during 70% MVC trials, YOUNG showed increased intermuscular coherence at 22–24 Hz and over several frequencies of approximately 36–64 Hz ($P < 0.05$, **Figure 6C**). Sporadic significant, but non-systematic, increases were observed in some frequency bins during 100% MVC in YOUNG (not shown). OLDER showed no training-induced changes in coherence level during any contraction intensity.

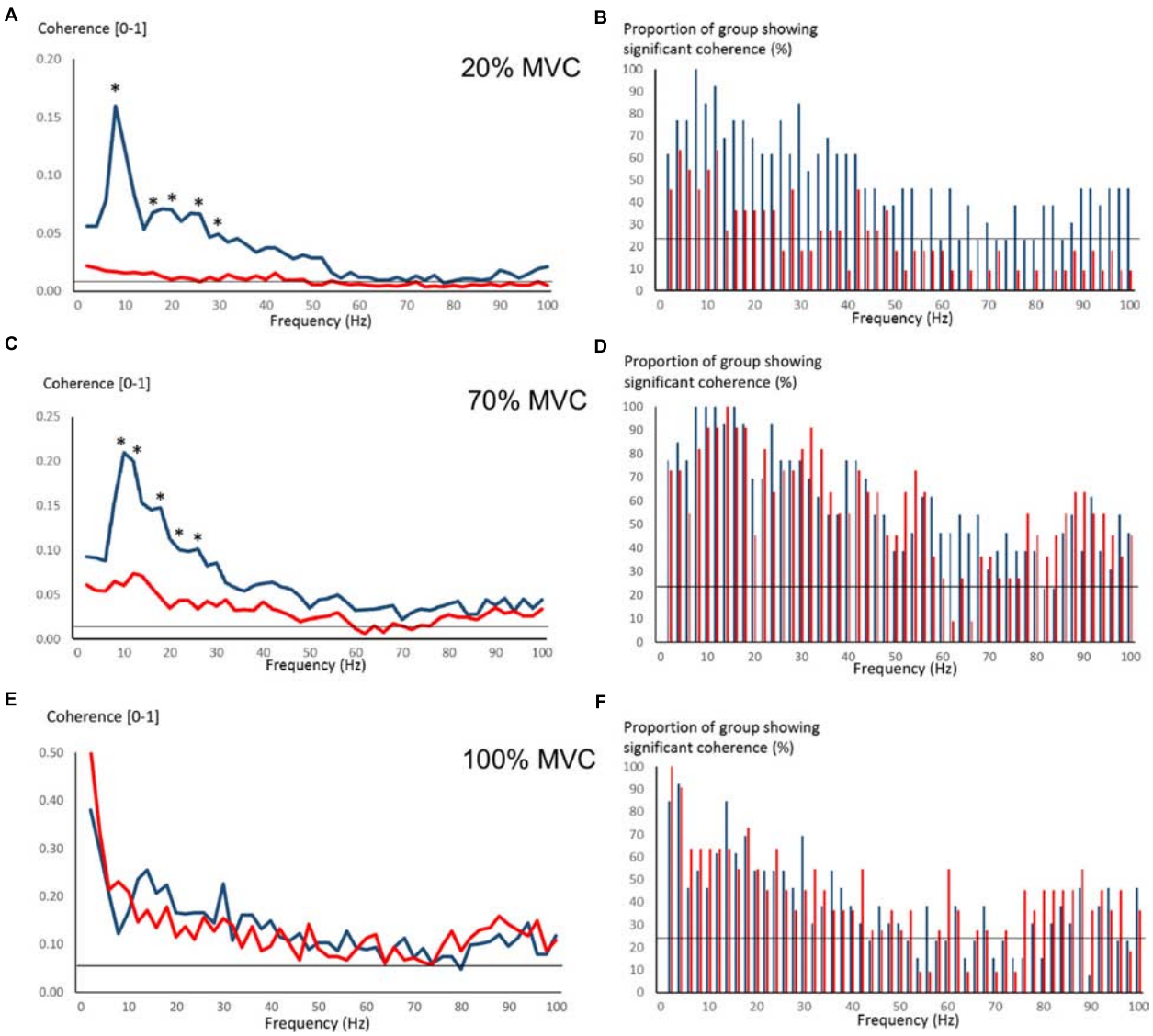

FIGURE 4 | Pre-training intermuscular coherence over 0–100 Hz during 20% **(A)**, 70% **(C)**, and 100% **(E)** MVC trials. Data are averaged for each age-group (YOUNG = blue color, OLDER = red color). *$P < 0.05$ YOUNG vs. OLDER based on Z-score analyses. The accompanying histograms for the three contraction intensities **(B,D,F)** show the proportion of subjects within each age-group demonstrating significant coherence for each frequency bin over 0–100 Hz. The horizontal lines in the figures represent the level of significance.

Significant main effects for time ($F = 8.5$, $P = 0.008$) and time × group ($F = 4.6$, $P = 0.043$) were observed in 40–60 Hz coherence during 70% MVC trials. YOUNG significantly increased coherence during 70% MVC trials within the 40–60 Hz band ($P = 0.008$, **Figure 7B**). During 100% MVC trials, a significant main effect for time × group ($F = 9.7$, $P = 0.005$) was observed in 8–14 Hz coherence. Here, OLDER significantly decreased 8–14 Hz band intermuscular coherence ($P = 0.003$, **Figure 7C**).

Body Composition Pre- and Post-training

There were no between-group differences in thigh fat mass, thigh fat-free mass or the change in these variables between-groups. A significant main effect for time ($F = 44.2$, $P < 0.001$) was observed in thigh fat-free mass. Thigh fat-free mass increased in YOUNG (6.4 ± 1.1 to 6.8 ± 1.3 kg, 6 ± 5%, $P = 0.001$) and in OLDER (5.5 ± 1.1 to 5.9 ± 1.1 kg, 7 ± 4%, $P = 0.001$) pre- to post-training.

Predicting Maximum Strength and Force Steadiness

Pre-training, a significant regression model was observed for 100% MVC torque ($F = 32.32$, $P < 0.001$) explaining 58% of the variance (adjusted $R^2 = 0.577$). The only significant variable within the model was thigh fat-free mass (beta 0.771, $P < 0.001$). For force steadiness, a significant regression model was observed

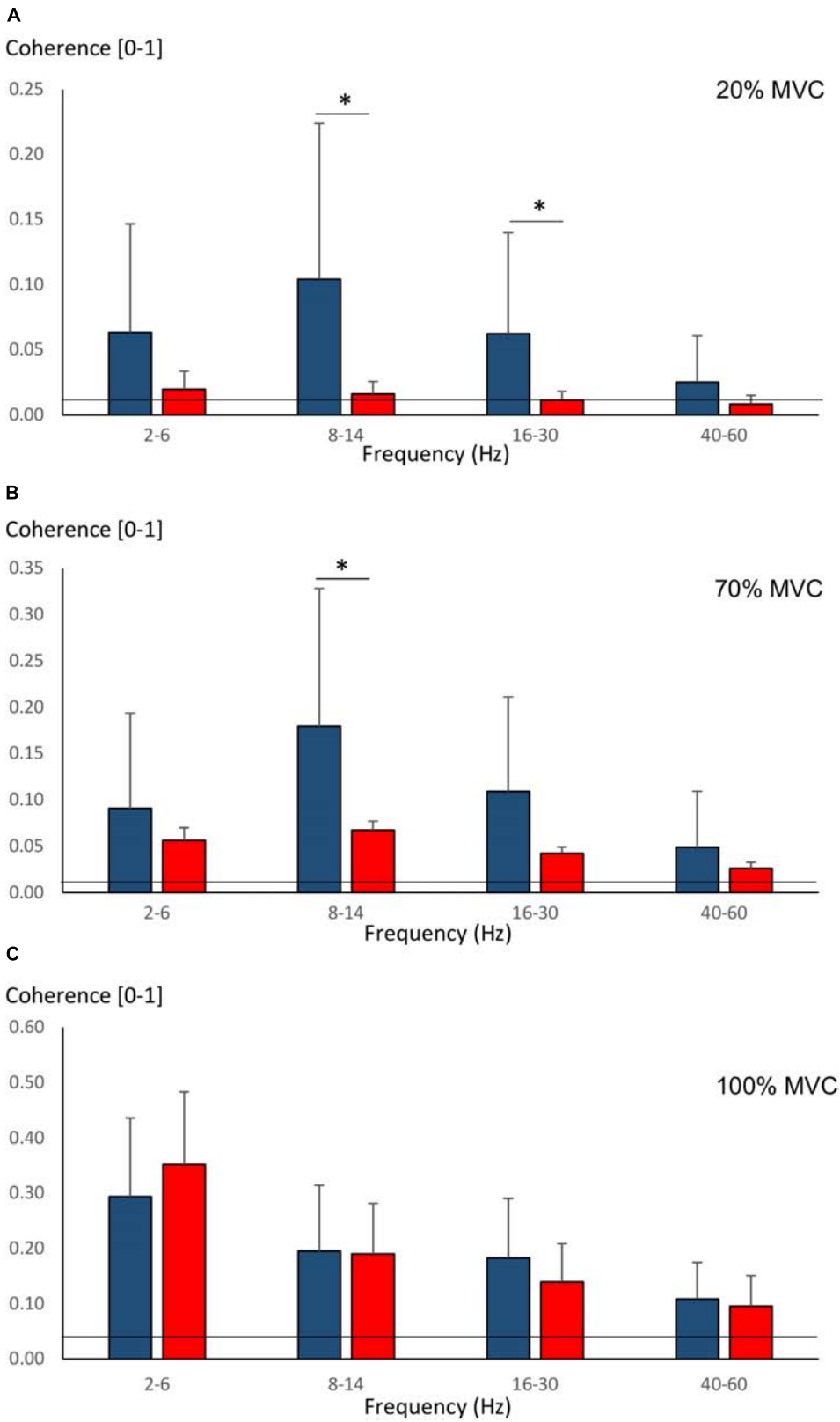

FIGURE 5 | Pre-training average intermuscular coherence (mean ± SD) within each studied frequency band in YOUNG (blue color) and OLDER (red color) during 20% **(A)**, 70% **(B)**, and 100% **(C)** MVC trials. *$P < 0.0125$.

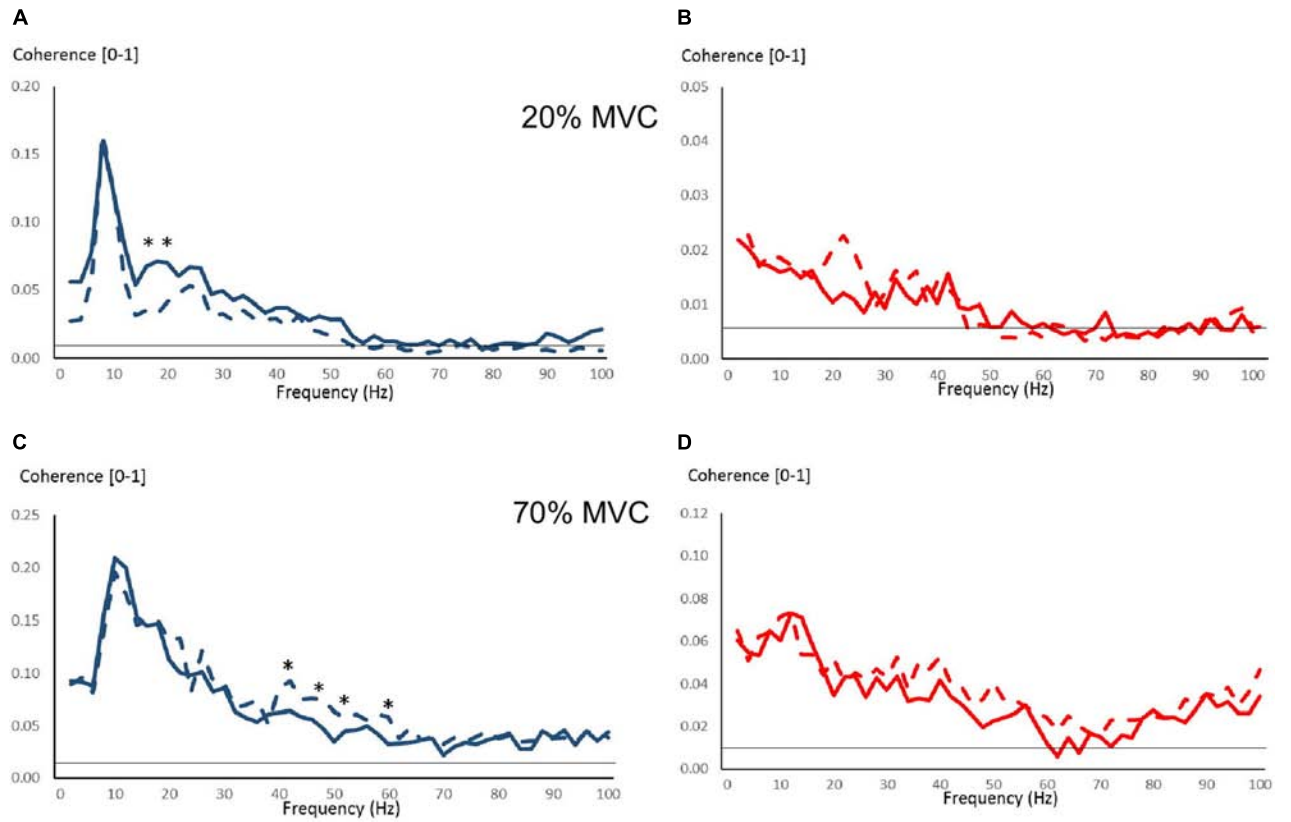

FIGURE 6 | Intermuscular coherence pre- and post-training over 0–100 Hz in YOUNG (blue color) and OLDER (red color) during 20% **(A,B)** and 70% **(C,D)** MVC trials. Data are averaged (Pre-training = solid lines, Post-training = dashed lines). The horizontal lines represent the level of significance for coherence. *$P < 0.05$ pre- vs. post-training based on Z-score analyses.

during 70% MVC trials explaining 40% of the variance ($F = 16.39$, $P = 0.001$, adjusted $R^2 = 0.401$). The only predictor was 100% MVC torque (beta 0.653, $P = 0.001$). Similar regression models were observed for post-training variables, but no significant models were observed for changes from pre- to post-training.

DISCUSSION

The present study demonstrated distinctive age-related differences in intermuscular coherence between young and older individuals. Broadly, young individuals demonstrated greater 8–14 and 16–30 Hz intermuscular coherence during 20 and 70% MVC trials. These results suggest differences in the function of the neuronal sensorimotor circuits between the age groups, despite their similar pre-training motor performances (i.e., maximum force production and force steadiness). During 70% MVC trials, young individuals displayed increased 40–60 Hz intermuscular coherence after the training period. However, regression analyses revealed that neither intermuscular coherence magnitude nor training-induced changes in intermuscular coherence could explain the variance in maximum force or force steadiness. Furthermore, YOUNG improved maximum force production to a greater extent than OLDER but showed no training-induced changes in coherence level during 100% MVC. Thus, our results cannot confirm functional significance of intermuscular coherence during maximum force production or force steadiness contractions of the knee extensors within the confinements of our study protocol.

Effects of Aging

Pre-training maximum isometric force (MVC) did not differ between YOUNG and OLDER in the present study. This may have been due to the subject composition of the two groups, with men accounting for 46% of OLDER and only 30% of YOUNG. Given that thigh fat-free mass was not different between groups, it would be doubtful that specific tension would differ greatly between the groups in our sample. Despite a lack of difference in maximum force production and subsequent force steadiness, YOUNG demonstrated greater intermuscular coherence during 20 and 70% MVC trials across 8–36 Hz frequencies and within 8–14 and 16–30 Hz bands when averaged. This supports the interpretation that difference in coherence reflected central, rather than purely peripheral processes.

Regarding intermuscular coherence within the 16–30 Hz band, it has been shown that the magnitude of coherence is dependent upon an intact (Fisher et al., 2012) and fully

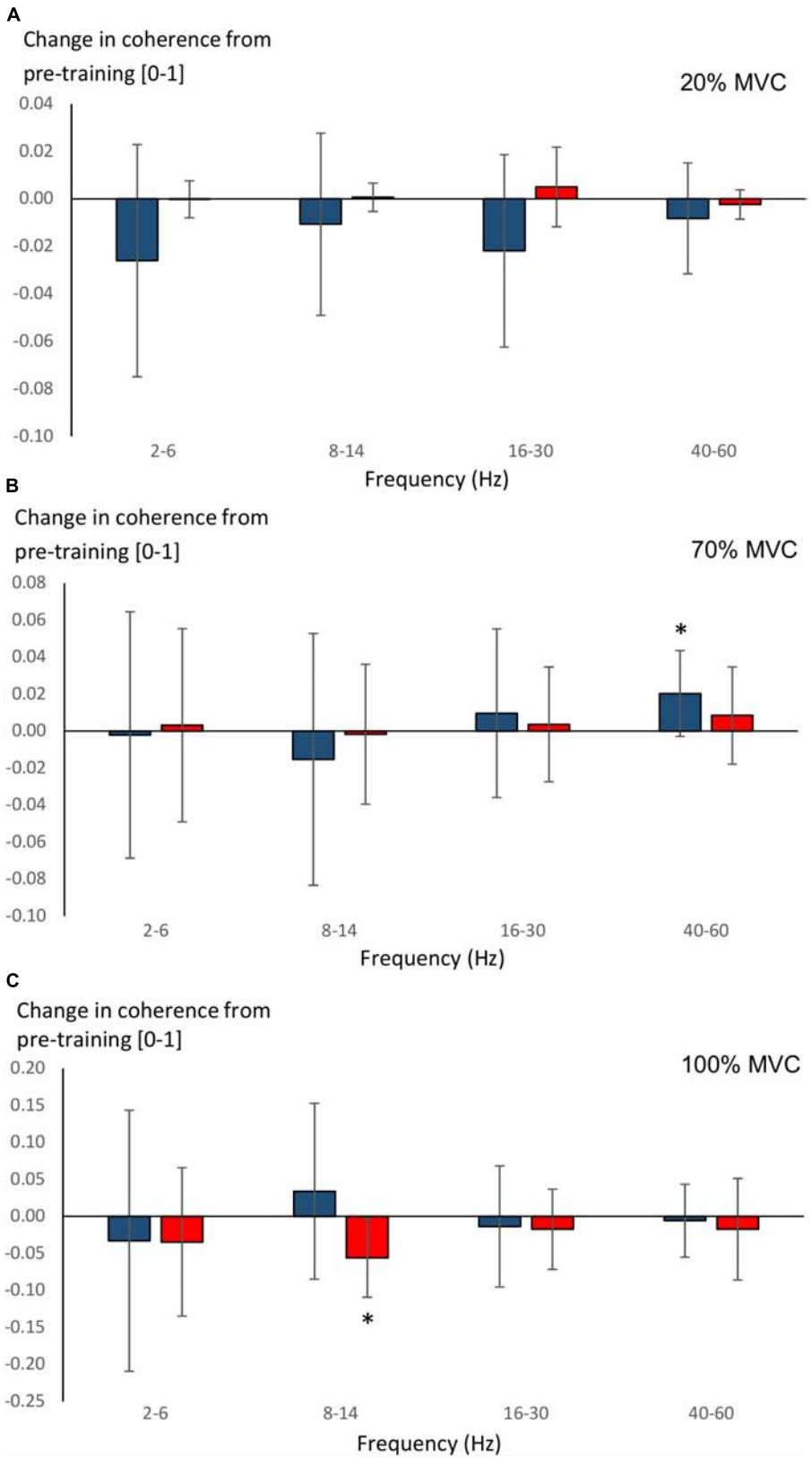

FIGURE 7 | Changes (Δ, mean $\pm$ SD) from pre-training in average intermuscular coherence within each studied frequency band in YOUNG (blue color) and OLDER (red color) during 20% **(A)**, 70% **(B)**, and 100% **(C)** MVC trials pre-training. $^*P < 0.0125$ within-group comparisons.

functional (Velazquez-Perez et al., 2017) corticospinal tract, and is reduced following acute stroke (Larsen et al., 2017). Consequently, coherence within the 16–30 Hz band can be considered to represent common cortical inputs to motoneurons via the corticospinal tract. The greater 16–30 Hz coherence in intermuscular of YOUNG supports this viewpoint, and suggests that OLDER had compromised corticospinal tract function or a reliance on other neural sites of modulation to regulate performance. For example, one possible reason for compromised corticospinal tract function would be the well-documented loss of myelinated corticospinal axons (Terao et al., 1994) as well as neurons throughout the central nervous system (Marner et al., 2003).

Our results are in direct contrast to the corticomuscular coherence results obtained by Kamp et al. (2013), who suggested that greater coherence was necessary during healthy aging as a means of counteracting loss of cortical functioning. However, subjects in the study by Kamp et al. (2013) performed pseudo-isometric wrist extension contractions. In needing to maintain a constant wrist position in a free environment, the subjects may have had to rely on proprioception to a greater extent than during a typical isometric contraction. We have recently shown that corticokinematic coherence, i.e., coherence between sensorimotor cortex (MEG) and foot acceleration signals during repetitive passive ankle rotations, is greater in older subjects likely due to inefficient cortical processing of proprioceptive afference (Piitulainen et al., 2018). It is important to note that different coherence measures along with varying experimental conditions reflect different aspects of sensorimotor processing/functioning. Thus, the findings should be discussed within the methodological constraints of that particular study.

The present study's lack of difference in force steadiness between age-groups is not entirely surprising considering that the muscle group tested was the knee extensors and that the lowest force level was 20% of MVC (Enoka et al., 2003). Typically, differences in force steadiness between young and older individuals would be observable more clearly in finger muscles and at force levels $\leq$10% of MVC (Enoka et al., 2003). Nevertheless, YOUNG demonstrated greater intermuscular coherence during our lowest contraction intensity (i.e., 20% MVC trials) within 8–14 and 16–30 Hz bands. Intermuscular coherence is thought to reflect common neural input to motoneurons from cortical, subcortical and spinal influences (Grosse et al., 2002). Therefore, the present study's findings suggest that aging reduces synchrony of common neural inputs to alpha-motoneurons.

While the source of 16–30 Hz coherence has been discussed above, intermuscular coherence around 10 Hz has been proposed to represent the level of Ia afferent feedback (Lippold, 1970; Erimaki and Christiakos, 2008). Previous studies have observed differences in the ability of young and older individuals to modulate force control via afferent feedback (Baudry et al., 2010; Holmes et al., 2015). Hence, it is plausible that our finding of lower 8–14 Hz intermuscular coherence during both 20 and 70% MVC trials in OLDER compared to YOUNG reflects a reduced ability to modulate force via afferent feedback. If older individuals have compromised afferent feedback, which is reflected in the level of 8–14 Hz intermuscular coherence, the question remains as to what mechanisms older individuals use to compensate for this in order to perform at a similar level to young. One other possibility is that older individuals had several common inputs within the 8–14 and 16–30 Hz frequency bands; the superimposition of these multiple sources could lead to lower coherence levels (Farina et al., 2014).

There are several limitations that should be acknowledged when utilizing intermuscular coherence measures. For example, when comparing between individuals, the EMG signals and thus its spectral content vary due to both anatomical and morphological reasons. Even individual motor unit action potentials have different shapes. One possibility is that electrodes placed close to the innervation zone may show lower coherence (Keenan et al., 2011). Innervation zone location can be highly variable along the muscle belly between individuals, and the zones are known to shift with force level even during isometric contractions (Piitulainen et al., 2009). Nevertheless, EMG locations were identified by SENIAM guidelines and they were as identical as possible for all individuals, hence, a systematic difference in placement relative to innervation zone seems unlikely, particularly since the focus of SENIAM is to place electrode between the musculotendinous junction and innervation zone. Further, one study showed no influence of electrode location relative to the innervation zone on corticomuscular coherence (Piitulainen et al., 2015). Hence, these potential influences are perhaps minor.

Another consideration is that signal-to-noise ratio can influence the level of coherence in noisy signals. In the case of intermuscular coherence, the EMG can be considered noisy, since the coherent EMG component is a relatively low proportion of the total EMG signal power. However, while there were increases in fat-free mass (i.e., muscle mass) due to training, there were no differences between groups. Furthermore, since no changes in fat mass of the thigh region were observed in either group, morphological changes leading to differences in signal-to-noise ratio are unlikely to play a major role in our results. Indeed, when measured, there were no differences in signal-to-noise ratio between the age groups in the present study (data not shown).

Effects of Strength Training

Maximum voluntary contraction increased as expected during the present study in both young and older individuals, although YOUNG gained more strength than OLDER due to the training. Most previous studies have shown equivalent strength gains between age-groups but some show greater gains in young people (Suetta et al., 2009; Greig et al., 2011). Therefore, our findings are not without precedent. Neither YOUNG nor OLDER improved steadiness during 20% MVC trials, and YOUNG reduced steadiness during 70% MVC trials. These results were unexpected since previous short-term strength training studies have observed improvements in steadiness in hand (Griffin et al., 2009) and knee extensor muscles (Tracy and Enoka, 2006). However, both YOUNG and OLDER showed remarkably low-force coefficient of variance (<1% at 20% MVC and <2% at 70% MVC) in comparison with the results of Tracy and Enoka (2006) already at pre-training. This would naturally reduce the

likelihood of identifying a possible functional role of coherence on force steadiness.

If it is assumed that intermuscular coherence at about 10 Hz is reflective of Ia afferent feedback (Lippold, 1970; Erimaki and Christiakos, 2008), then there appears to be no training-induced changes in pre- or post-synaptic inhibition in either YOUNG or OLDER in the present study. Previous strength training studies have not seen consistent increases in H-reflex amplitude of the lower limbs, with reports of both positive (Aagaard et al., 2002) and no effect (Vila-Cha et al., 2012). Limited evidence suggests that afferent feedback from the lower limb is not improved by short-term strength training in older individuals (Unhjem et al., 2015), which could be of functional importance (e.g., in balance control). Nevertheless, it is difficult to ascertain possible mechanisms of adaptation.

An interesting finding is that 40–60 Hz coherence increased after training in YOUNG during 70% MVC trials but not in 100% MVC trials. The so-called Piper rhythm has been observed during high-force contractions (Piper, 1907) and has been observed in corticomuscular coherence studies (Brown et al., 1998), although not all lower limb muscles demonstrate a shift toward higher frequencies (Ushiyama et al., 2012). One feature that distinguished the 70% MVC trials from the 100% MVC trials in the present study was the intention to maintain force steadiness. During the 100% MVC trials, the subjects were instructed to exert their maximum force, and were not interested in keeping to a specific target. In this regard, there may be some other factor responsible for the training-induced increase in 40–60 Hz coherence observed during 70% MVC trials rather than simply a greater contraction force. It has been suggested that heightened attentiveness may possibly affect cortical Piper activity (DeFrance and Sheer, 1988; Brown et al., 1998). Since YOUNG showed worsening of force steadiness during 70% MVC trials, which were related to the magnitude of increased maximum strength, this group may have experienced adaptations that allow/require greater attentiveness to a high-force steadiness task.

CONCLUSION

The present study identified clearly stronger intermuscular coherence in healthy young than older individuals during both low- and high-force isometric knee extension contractions, particularly over 8–40 Hz frequencies. A short-term strength training intervention increased the coherence strength during 70% MVC trials in young individuals (40–60 Hz) and reduced coherence strength during 100% MVC trials in older (8–14 Hz) individuals. It appears that enhanced 40–60 Hz coherence does not solely reflect the degree of force production capacity, since coherence level increased in YOUNG during 70% but not 100% MVC trials after training. Finally, the functional significance of intermuscular coherence level remains unclear, since neither the performance measures (i.e., force steadiness and maximum force) or their training-induced changes associated with the coherence/changes in coherence strength of any studied frequency band.

AUTHOR CONTRIBUTIONS

SW, JA, JW, RM, HP, and TP conceived and designed the study. SW performed the experiments. SW and SB analyzed the data. SW, RM, HP, and SB interpreted the results. SW and SB prepared the figures. SW drafted the manuscript. SW, JA, JW, RM, HP, SB, and TP edited and approved the final manuscript.

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Lifelong Football Training: Effects on Autophagy and Healthy Longevity Promotion

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Aging is a physiological process characterized by a progressive decline of biological functions and an increase in destructive processes in cells and organs. Physical activity and exercise positively affects the expression of skeletal muscle markers involved in longevity pathways. Recently, a new mechanism, autophagy, was introduced to the adaptations induced by acute and chronic exercise as responsible of positive metabolic modification and health-longevity promotion. However, the molecular mechanisms regulating autophagy in response to physical activity and exercise are sparsely described. We investigated the long-term adaptations resulting from lifelong recreational football training on the expression of skeletal muscle markers involved in autophagy signaling. We demonstrated that lifelong football training increased the expression of messengers: RAD23A, HSPB6, RAB1B, TRAP1, SIRT2, and HSBPB1, involved in the auto-lysosomal and proteasome-mediated protein degradation machinery; of RPL1, RPL4, RPL36, MRLP37, involved in cellular growth and differentiation processes; of the Bcl-2, HSP70, HSP90, PSMD13, and of the ATG5-ATG12 protein complex, involved in proteasome promotion and autophagy processes in muscle samples from lifelong trained subjects compared to age-matched untrained controls. In conclusion, our results indicated that lifelong football training positively influence exercise-induced autophagy processes and protein quality control in skeletal muscle, thus promoting healthy aging.

Keywords: football, lifelong training, longevity, cardiovascular capacity, autophagy

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INTRODUCTION

Aging is a physiological process characterized by a progressive decline of biological functions and a progressive increase of destructive processes in cells and organs, eventually leading to death (López-Otín et al., 2013; Aunan et al., 2016; Flatt and Partridge, 2018).

This process not only affects the homeostasis within organs and the tissue response to injury, but, at a molecular level, it is associated with accumulation of DNA and protein damage (Soares et al., 2014; Boros and Freemont, 2017). Several physiological changes occur with aging, the most

notable are: the decrease of cardiac output at rest and maximum breathing capacity, decrease in renal filtration rate and in nerve conduction velocity (McClaran et al., 1995; Glassock and Winearls, 2009; Strait and Lakatta, 2012; Jafari Nasabian et al., 2017). Additionally, aging acts also on the metabolically active bone and muscle tissues by triggering events that eventually impair their cross-talk determining low body mass density (BMD) and/or sarcopenia (Boirie, 2009). At this regard, fatty infiltration in both tissues is considered an age-related hallmark leading to osteosarcopenia, a recently defined geriatric syndrome, which is associated to increased morbidity and mortality risk among older people (Hirschfeld et al., 2017). Among the shared patho/physiological pathways, bone and muscle tissues can both adapt to mechanical stimuli, these latters being able to regulate the delicate balance between osteogenesis and osteolysis and between muscle growth and breakdown (Isaacson and Brotto, 2014; Kawao and Kaji, 2015). Nowadays, it is well recognized the beneficial impact of physical activity on multiple organ functions, by providing an adeguate and efficient mechanical stimulus on all the components of the locomotor apparatus (Radak et al., 2018). Physical Activity (PA) and exercise training delays functional decline in most of the biological pathways in older people, and plays a key role in promoting healthy longevity (Booth et al., 2012; Pedersen and Saltin, 2015). PA and exercise has been shown to affect the skeletal muscle expression of markers involved in longevity pathways differently depending on the exercise mode. As such, it has been shown that long-term aerobic training increased the expression/activity of SIRT1 and SIRT3 in older rats (Ferrara et al., 2008; Palacios et al., 2009), whereas resistance training increased the expression of Heat Shock Proteins (HSPs) in rat skeletal muscle (Murlasits et al., 2006). Furthermore, the expression of HSPs in skeletal muscle following exercise induced stress appears to be intensity dependent, with higher exercise intensities producing a greater physiological response (Gjøvaag and Dahl, 2006; Folkesson et al., 2008; Paulsen et al., 2009, 2012). Also, HSPs induction is requested for the maintenance of cellular homeostasis and promotion of longevity (Lindquist and Craig, 1988; Hartl, 2016).

Recently, a new mechanism, autophagy, was indicated in the adaptation induced by acute and chronic exercise as being responsible for positive metabolic adaptation and longevity promotion (Vainshtein and Hood, 2016). Autophagy is an ubiquitous catabolic process which leads to the degradation of cytoplasmic components in the cells. This process plays a crucial role in the physiological turnover of most proteins, biological membranes, mitochondria, and ribosomes (Park and Cuervo, 2013). Autophagy impairment may ultimately lead to the accumulation of damaged cellular components, including mitochondria and protein aggregates and is associated to the aging process both in invertebrates and higher organisms (Cuervo et al., 2005; Terman and Brunk, 2006; Schiavi and Ventura, 2014; Sakuma et al., 2017).

The acute physiological response to a recreational football training session in elderly subjects is characterized by a high aerobic load, with mean and peak heart rate (HR) reaching 84–88% and 93–98% of individual maximum heart rate (HRmax), respectively (Randers et al., 2010; Andersen et al., 2014). Also, during a recreational football training session in elderly subjects, significantly elevated blood lactate levels have been reported (Andersen et al., 2014). This finding supports that lactate production is high during a football training session, and that the intense and frequently changing activity pattern observed during a football training session activates glycolysis to a greater extent than continuous walking and straightforward running performed at similar or higher average movement speed (Drust et al., 2000). The above findings are comparable to what can be observed in elite football players (Krustrup et al., 2006).

Long-term recreational football training increases skeletal muscle fat oxidation and anti-oxidative potential, stimulates musculoskeletal metabolic adaptations, and results in beneficial improvements in the cardiovascular system (Krustrup et al., 2010; Alfieri et al., 2015; Bangsbo et al., 2015; Schmidt et al., 2015; Andersen et al., 2016; Krustrup and Krustrup, 2018). However, up to now, the molecular effects of exercise training on regulation of autophagy and processes involved in longevity promotion are not completely elucidated, and no consensus is established about the different gene/protein expression in response to exercise (Koltai et al., 2012; Kim et al., 2013; Konopka et al., 2014; Vainshtein et al., 2014; Moreira et al., 2017).

Very recently, increased expression of key markers involved in mitochondrial biogenesis, oxidative metabolism, and DNA-repair/senescence suppression pathways were found in skeletal muscle from Veteran Football Players (VPG) compared to untrained elderly subjects (Mancini et al., 2017). As such, the aim of the present research was to investigate, through a differential transcriptomic approach, the effects of lifelong recreational football training on the expression of key markers involved in the autophagy response for the maintenance of protein quality control, related to healthy longevity promotion, in skeletal muscle from VPG compared to elderly untrained control subjects.

MATERIALS AND METHODS

Subjects

Thirty healthy males aged 65–77 years volunteered to take part of the study. The subjects were divided into two groups according to their previous experience as football players, with one group consisting of veteran football players (VPG; $N = 15$), and one group consisting of healthy age-matched untrained controls (CG; $N = 15$) (anthropometric and clinical characteristics are reported in **Table 1**). VPG was recruited via direct contact to local football clubs in the greater Copenhagen area, and had on average been active as football players for 52 $\pm$ 11 years (median 58 years, range 25–62 years) and had been training one session per week (1.5 $\pm$ 0.6 h/session) and played 26 $\pm$ 12 football matches (2 $\times$ 35 min) per year for the last 10 years as previously reported (Schmidt et al., 2015). CG was recruited via advertisement in local newspapers, and none of the subjects had been involved in regular physical exercise training during a major part of their adult life. In addition, the participants reported that they had been primarily inactive for the past 5–10 years.

TABLE 1 | Anthropometric and clinical characteristics of subjects participating to the study.

	VPG	CG
Number of subjects	15	15
Age (yrs)	69.3 ± 3.2	68.3 ± 2.8
Height (cm)	178.5 ± 4.9	176.8 ± 6.3
Body weight (kg)	77.4 ± 7.7	85.9 ± 11.2*
BMI (kg/m^2)	24.3 ± 2.2	27.5 ± 3.7*
Total body fat (%)	22.9 ± 6.7	30.8 ± 4.6*
VO_2peak (mL/kg/min)	33.7 ± 5.4	27.6 ± 4.9*

*$p < 0.05$ VPG vs CG. Values are reported as means±SDs.

All subjects were informed verbally and in writing about any potential discomforts or risks related to the experimental study protocol, and signed an informed written consent prior to their enrolment in the study. For both groups, inclusion criteria were eligibility to all testing procedures (see below). Exclusion criteria were history or symptoms of cardiovascular disease or cancer, type 2 diabetes, hypertension, nephropathy, or musculoskeletal complaints that were considered to preclude testing. All medical screening procedures were performed by a medical doctor. The study was conducted according to the Declaration of Helsinki and was approved by the local ethical committee of Copenhagen; H-1–2011-013. ClinicalTrials.gov identifier: NCT01530035.

Habitual Fitness Level

To estimate the daily physical activity level of all participants in CG and in VPG besides the football training sessions and matches, participants filled a questionnaire to quantify all sporting activities and everyday activities. Physical activity was defined as hours spent walking, jogging, running, swimming, biking, golfing, or gardening in order to relate to current standards of advised physical activity in elderly citizens. In VPG, nine subjects did not participate in other sporting activities besides football, whereas the remainder reported activities for 2.0 ± 1.5 h/week besides football training consisting of golf, running, biking, fitness, or kayaking. Additional physical activities or no additional physical activities besides football training in VPG were not associated with higher VO_2peak values as previously reported (Schmidt et al., 2015). In CG, the subjects were on average active 2.5 ± 3.1 h/week (median 2 h, range 0–10 h/week) mainly consisting of everyday activities such as housing and gardening, gymnastics, walking, golf, swimming, and no activities. Thus, the majority of the physical activities in CG did not amount to a formal moderate-intensity aerobic level[1]. Two subjects in CG reported more than 5 h of physical activity but were active with walking and golf, i.e., low-intensity activities. As such, in CG, a large variance in habitual physical activity levels was observed. However, habitual physical activity in CG was not significantly associated with higher cardiorespiratory fitness (Schmidt et al., 2015).

[1]https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/213741/dh_128146.pdf

Muscle Sample Collection

Muscle biopsies were obtained under standardized conditions after an overnight fast between 7 and 10 a.m. from the vastus lateralis under local anesthesia (1% Lidocaine, Amgros 742122, Copenhagen, Denmark) using the Bergstrom technique. The muscle sample (40 mg wet weight) was immediately frozen in liquid N_2 and stored at −80°C until further analysis. All subjects were instructed to refrain from strenuous physical activity or exercise training 48–72 h prior to the invasive procedure in addition to consuming a standardized carbohydrate-rich meal the night before reporting to the laboratory as previously described (Andersen et al., 2016).

Body Composition

Whole body fat percentage was determined by whole body Dual energy X-ray absorptiometry (DXA) scanning (Prodigy Advance, Lunar Corporation, Madison, WI, United States). Scanning was performed between 7 and 10 a.m. under standardized conditions after an overnight fast. All DXA scans were performed by the same experienced observer and the DXA software regional cut-points were visually inspected and manually adjusted if necessary. Body height and body weight were measured on standard scales with subjects wearing light clothes and BMI (kg/m^2) was subsequently calculated.

Peak Oxygen Uptake

For determination of peak oxygen uptake (VO_2peak) an incremental cycling test to exhaustion was applied. During the incremental cycling test, subjects started exercising at a work pace and load of 80 rpm and 40 W, respectively, after which the work load was increased by 20 W every 2-min until volitional fatigue. Pulmonary gas exchange (OxyconPro; VIASYS Healthcare, Höechberg, Germany) was measured continuously throughout the exercise protocol. VO_2peak was determined as the highest value achieved during a 30-s period.

Transcriptome Assay in Muscle Biopsies Samples

RNA extraction has been performed as previously described (Mancini et al., 2017). Total RNA was extracted from the muscle biopsies using an miRNeasy® kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The RNA integrity number (RIN) of samples was assessed using a Bio-Rad Experion automated electrophoresis station (Hercules, CA, United States) before cDNA synthesis. All RNA samples passed the criterion of RIN ≥7. For strong, unbiased results, pooled RNA libraries were produced by evenly pooling six RNA samples, which resulted in three pooled CGs (named CG1, CG2, and CG3) and three pooled VPG libraries (named VPG1, VPG2, and VPG3). The pooled RNA samples underwent microarray assay to determine gene expression profile. In this study, we carried out profiling with GeneChip® Human Transcriptome Array 2.0 (HTA 2.0, Affymetrix, Santa Clara, CA, United States). The RNA samples were prepared using the WT PLUS Reagent kit, followed by hybridization on HTA 2.0 microarray chips. 100 ng of total RNA were subjected

to two cycles of cDNA synthesis with the Affymetrix WT PLUS expression Kit. The first cycle (first strand synthesis) is performed using an engineered set of random primers that exclude rRNA-matching sequences and include the T7 promoter sequences. After second-strand synthesis, the resulting cDNA is *in vitro* transcribed with the T7 RNA polymerase to generate a cRNA. This cRNA is subjected to a second cycle – first strand synthesis in the presence of dUTP in a fixed ratio relative to dTTP. Single strand cDNA is then purified and fragmented with a mixture of uracil DNA glycosylase and apurinic/apyrimidinic endonuclease 1 (Affymetrix) in conjunction with incorporated dUTPs. DNA fragments are then terminally labeled by terminal deoxynucleotidyl transferase (Affymetrix) with biotin.

The biotinylated DNA was hybridized to the Human Genechip HTA 2.0 Arrays (Affymetrix), containing more than 285.000 full length transcripts covering 44.700 coding genes and 22.800 non-coding genes selected from H. sapiens genome databases RefSeq, ENSEMBL, and GenBank. Chips were washed and scanned on the Affymetrix Complete GeneChip® Instrument System, generating digitized image data (DAT) files. The data discussed in this publication have been deposited in NCBI's Gene Expression Omnibus (Edgar et al., 2002) and are accessible through GEO Series accession number GSE125830[2].

Bioinformatic Analysis

Genomic data were subjected to Database for Annotation, Visualization and Integrated Discovery (DAVID)[3] and Ingenuity Pathways Analysis (IPA) (Ingenuity System[4]) to identify and explore relevant biological networks. Genes were uploaded as a tab-delimited excel file of Gene Symbol and Fold Change and mapped to corresponding gene objects stored in the IPA.

RNA Extraction and RT*q*PCR

Total RNA was extracted from the muscle biopsies and integrity assessed as described above. For reverse transcription-PCR (RT-PCR) analysis, 0.5 μg of total RNA was used in the reaction with SuperScript reverse transcriptase (Life Technologies). The resulting cDNA was analyzed by realtime quantitative PCR (RTqPCR) performed with the iQ5- iCycler Optical System (Bio-Rad, Hercules, CA, United States). IQ SYBR Bio-Rad protocol (100 mM KCl, 40 mM Tris–HCl pH 8.4, 0.4 mM dNTPs, iTaq DNA polymerase 50 U/ml, 6 mM MgCl2, SYBR Green I, 20 nM fluorescein, stabilizer) was applied according to the manufacturer's instructions. Reaction mixtures were incubated at 95°C for 30 s, followed by two cycles at 95°C for 30 s and 95°C for 3 min and by 40 cycles at 95°C for 15 s and 60°C for 1 min. Finally, 80 cycles were run starting at 55°C and increasing the temperature by 5°C every 10 s up to 95°C. Fluorescence signals were measured during the elongation step. All measurements were performed in duplicate. The target mRNA expression levels were normalized to the levels of the polymerase (RNA) II (DNA directed) polypeptide A (Pol2A) gene using the $2^{-\Delta\Delta CT}$ method as described in (Vitucci et al., 2018). The Oligonucleotide primer sequences used in RTqPCR are reported below:

RPLP1 Fw	GCCCTCATTAAAGCAGCCG
RPLP1 Rev	CAAAAAGACCAAAGCCCATG
RPL4 Fw	TGTCTAAAGGTCATCGTATTG
RPL4 Rev	GCGACGGTTTCTCATTTTGC
RPL36 Fw	CAAGTTCGTGCGGGACATG
RPL36 Rev	GGCTCAGTCTTTCTTGGCAG
MRPL37 Fw	CGGTATGGCATTGGCGTCC
MRPL37 Rev	GCAAAGGTGATGGGCTCC
RAB1B Fw	CGCCATGAACCCCGAATAT
RAB1B Rev	CTGAAGTTTGATAGTTTTGCC
SIRT2 Fw	GCTCAGGACTCAGATTCAGA
SIRT2 Rev	TCCCACCAAACAGATGACTC
TRAP1 Fw	GCAGGGTTCCACTTCCAAA
TRAP1 Rev	GTGATGGTGCCTTTCTCGG
RAD23A Fw	GAGACGGTGAAGGTGCTAAA
RAD23A Rev	TCTGGGGGTGCTGAGGTAC
HSPB6 Fw	GCTGTCAAGGTGGTGGGC
HSPB6 Rev	CCTCCTACTTGGCTGCGG
HSPB1 FW	CCTGAGGGCACACTGACC
HSPB1 Rev	AGGCTTTACTTGGCGGCAG

Western Blotting

The muscle biopsies were mechanically pulverized and protein extraction was performed as previously described (Mancini et al., 2017). Briefly, protein samples (50 μg each) were separated on 4–20% precast gradient polyacrylamide gels (Bio-Rad), transferred to the Hybond ECL nitrocellulose membrane (GE Healthcare) and checked by Ponceau S staining to verify equal loading. The membranes were immunoblotted using rabbit polyclonal antibodies against autophagy related 5 homolog (ATG5), autophagy related 12 homolog (ATG12), mouse monoclonal antibodies against Heat Shock Protein 90 (HSP90), Heat Shock Protein 70 (HSP70), B-cell lymphoma 2 (Bcl-2) (Elabscience, 1:500), monoclonal rabbit antibody proteasome 26S subunit non-ATPase 13 (PSMD13) (Abcam, 1:1000), monoclonal mouse antibody against glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (1:1000; Santa-Cruz Biotechnology Inc.). Blots were incubated with appropriate horseradish peroxidase-conjugated secondary antibody and target proteins were visualized by ECL detection (GE Healthcare). Densitometric measurements were carried out using Quantity One software (Bio-Rad) as reported elsewhere (Imperlini et al., 2015). GAPDH protein was used to estimate the total amount of loaded proteins. Results were normalized as a percentage of the mean of controls in each membrane.

Statistical Analysis

Group comparisons were investigated with the application of a one-way ANOVA statistical model.

Microarray data analysis: DAT files were analyzed by Expression Console (Affymetrix Inc.). The full data set was

[2]https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE125830

[3]https://david.ncifcrf.gov/

[4]http://www.ingenuity.com

normalized by using the Robust Multi alignment Algorithm (RMA). The obtained expression values were analyzed by using the Affymetrix Transcription Analysis Console (TAC) software (Applied Biosystem – Thermo scientific-Italy). Further normalization steps included a per chip normalization to 50th percentile and a per gene normalization to median. Results were filtered for fold change >1.5. Statistical analysis was performed using the ANOVA using as p-value cutoff 0.05 and 0.0, respectively. Relative mRNA expression was reported as relative quantitation (RQ) values, calculated as $2^{-\Delta\Delta Ct}$, where ΔCt is calculated as $Ct_{\text{target gene}}$ - $Ct_{\text{housekeeping}}$ genes (PolR2A mRNA expression). Differences between VPG *vs* CG were considered statistically significant at $p < 0.05$. We used one-way ANOVA calculated with StatView software (version 5.0.1.0; SAS Institute Inc., Cary, NC, United States). Relative protein abundance of ATG5, ATG12, HSP90, HSP70, Bcl-2, and PSMD13 was calculated with respect to GAPDH protein abundance and analyzed with the ANOVA calculated with StatView software (version 5.0.1.0; SAS Institute Inc., Cary, NC, United States).

RESULTS

Identification of Differently Expressed Genes (DEGs) in Skeletal Muscle From Veteran Football Players (VPG) Compared to Untrained Subjects (CG)

We identified the DEGs in skeletal muscle from VPG compared to CG subjects by a GeneChip analysis. After data preprocessing, a total of 430 ($p < 0.05$) and 190 genes ($p < 0.01$), respectively, were identified as differentially expressed between groups. The gene list was further analyzed and a cluster heat map was generated (**Figure 1**).

Biological Network Analysis

Gene Ontology (GO) Analysis

To understand the function of DEGs, we performed a GO analysis, a commonly used approach for functional studies of large-scale genomic or transcriptomic data. GO terms include biological processes (BP), molecular function (MF), and cellular component (CC). GO analyses were conducted using the on-line software DAVID[5]. The significant enriched GO terms ($p < 0.05$) are listed in **Table 2**. We identified different GO terms, including 11 biological processes: tricarboxylic acid cycle (GO:0006099), ATP metabolic process (GO:0046034), structural constituent of ribosome (GO:0003735), 4 iron, 4 sulfur cluster binding (GO:0051539), cellular carbohydrate catabolic process (GO:0044275), sarcomere (GO:0030017), nicotinamide metabolic process (GO:0006769), negative regulation of transcription factor activity (GO:0043433), striated muscle contraction (GO:0006941), intracellular organelle lumen (GO:0070013), mitochondrial respiratory chain (GO:0005746).

[5]https://david.ncifcrf.gov/

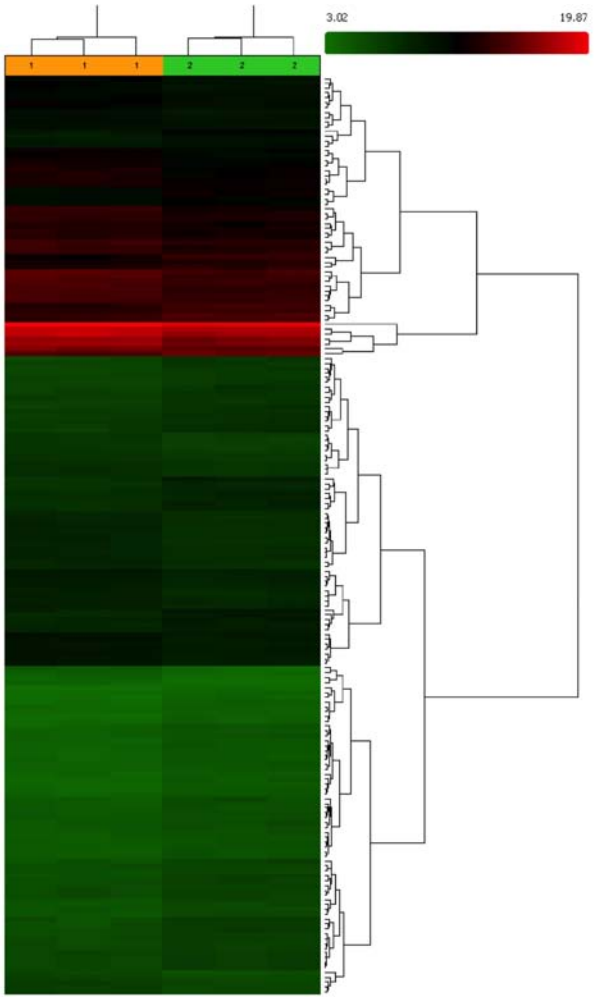

FIGURE 1 | Clustering output of DEGs in skeletal muscle of Veteran Football Players (VPG) compared to untrained (CG) subjects. In Red are up-regulated and in green down-regulated DEGs. A list of transcript identified, fold-change and gene identification is shows in **Supplementary Table S1**.

Biological Network Identification Affected by Lifelong Football Training in Muscle From VPG by Ingenuity Pathway Analysis (IPA)

To define biological networks affected by lifelong football training, we analyzed the 430 transcripts differentially expressed between the two groups ($p < 0.05$) using the IPA software. IPA output revealed five high-score multidirectional interaction networks. Among them one network was associated with "Small molecule biochemistry, Nucleic acid and amino acid metabolism" (score 79), another one with "Organ Morphology, Nervous System Development and Function" (score 51), and others with "Skeletal and Muscular System Development and Function" (score 25).

The results showed that identified DEGs belong to pathways that are involved in different processes correlated to healthy longevity, in particular, Nucleic Acid, amino acid and protein metabolism and Organ Morphology, Nervous

TABLE 2 | Enriched GO terms in biological processes.

GO ID	Gene Name	Count	*p*-value	RG	FDR	Enrichment
GO:0006099	Tricarboxylic acid cycle	5	2.906646E-6	ACO2, CS, IDH2, IDH3B, MDH2	0.000043	6.493506
GO:0046034	ATP metabolic process	6	9.960286E-5	ATP5D, ATP5G2, MYH7, ATP1A2, ATP5I, ATP6V1F	0.001498	7.792207
GO:0003735	Structural constituent of ribosome	6	9.734934E-4	RPL18A, RPLP1, RPL36, MRPL37, RPS9, RPL4	0.012611	7.792207
GO:0051539	4 iron, 4 sulfur cluster binding	3	0.005695	ACO2, NDUFV1, ETFDH	0.071725	3.896103
GO:0044275	Cellular carbohydrate catabolic process	4	0.006330	GPD1L, DLAT, OGDH, MDH2	0.091176	5.194805
GO:0030017	Sarcomere	4	0.009943	TCAP, ANKRD23, MYH7, CACNA1S	0.112608	5.194805
GO:0006769	Nicotinamide metabolic process	3	0.013542	IDH3B, DCXR, MDH2	0.185566	3.896103
GO:0043433	Negative regulation of transcription factor activity	3	0.016946	THRA, PRDX2, COMMD7	0.226872	3.896103
GO:0006941	Striated muscle contraction	3	0.017667	TCAP, MYH7, CACNA1S	0.235357	3.896103
GO:0070013	Intracellular organelle lumen	15	0.025394	ATP5D, ACO2, SRL, CS, RPS9, RPL36, IDH3B, PDLIM1, MYH7, DLAT, OGDH, GOT2, MRPL37, MDH2, VPS25	0.264731	19.480510
GO:0005746	Mitochondrial respiratory chain	3	0.034088	UQCRC1, NDUFB7, NDUFV1	0.339430	3.896103

GO, Gene Ontology; RG, related genes; FDR, false discovery rate.

System Development and Function (**Figures 2A,B**). The quantitative analysis by RT-*q*PCR confirmed the over-expression of messengers significantly enriched in the above-mentioned pathways in muscle from VPG. In particular, we found significant up-expression of RAD23A, HSPB6, RAB1B, TRAP1, SIRT2 ($p < 0.01$), HSPB1 ($p < 0.05$) messengers, which are involved in the auto-lysosomal and proteasome-mediated protein degradation machinery and maintenance of protein quality control and similarly, significant up-expression of RPLP1, RPL4, RPL36, MRLP37 ($p < 0.05$) messengers that are involved in cellular growth and proliferation in skeletal muscle from VPG compared to CG (**Figure 3**).

Lifelong-Football Training Affects the Muscle Expression of Rotein Markers Involved in Protein Quality Control Processes

To evaluate the effects of lifelong football training on the expression of key proteins involved in proteasome promotion and autophagy, we compared the expression of HSP70, HSP90, ATG5-ATG12 complex, Bcl-2, and PSMD13 proteins in muscle biopsies from VPG *vs* CG subjects. We found significant enhanced expression of all these proteins in muscle from Veterans compared to CG ($p < 0.05$), as reported in **Figure 4**.

DISCUSSION

Aging leads to accumulation of damage in functional protein complexes involved in essential cellular processes (Kirkwood, 2002; Wedel et al., 2018). Recently, it has been seen that during aging, an increase in protein damage can be exacerbated by reduced levels of heat shock proteins (HSP) and a diminished autophagy response with consequent impairment of protein quality control (Calderwood et al., 2009).

To our knowledge few studies have investigated the effects of acute and chronic exercise in the elderly on the molecular expression of key markers involved in longevity and on the levels of damaged proteins or organelles in skeletal muscle (Hood et al., 2011; Lira et al., 2013; Cheng et al., 2016; Dickinson et al., 2017; Radak et al., 2018). Autophagy and healthy aging is a new field of research that has not been fully explored until now. This is the first report that investigates the effects of lifelong football training on the autophagy process in particular on the protein biogenesis and anti-apoptotic pathway in skeletal muscle in elderly subjects. We demonstrated that messengers associated with auto-lysosomial and proteasome-mediated pathways were significantly up-regulated in skeletal muscle from VPG compared to CG. In particular we found up-expression of RPL4 and RPL36 messengers, which are regulators of cell-growth and differentiation, promotion of protein quality control and anti-apoptosis pathways in VPG muscle (Blount et al., 2014; Bareh et al., 2016). This is an important finding, as mutations and polymorphisms in RPLs genes are associated with DNA damage and apoptosis in different cells (Wadhwa et al., 2015).

TRAP1 messenger codify for a chaperone protein involved in the reprogramming of cell metabolism following any type of stress. Furthermore, TRAP1 is the only chaperone that directly interacts with mitochondrial respiratory chain components, and also, its expression is correlated to the respiratory capacity of the cells (Lisanti et al., 2014; Matassa et al., 2016). Accordingly, we found the expression of TRAP1 messenger to be up-regulated in VPG compared to CG subjects, which is in line with the improved cardiovascular capacity (VO_2max) reported for this group (Schmidt et al., 2015). Sirtuins are molecules that connect energy metabolism, oxidative stress, and aging (Karvinen et al., 2016; Zhang et al., 2016). SIRT2 regulates muscle gene expression and differentiation in response to exercise, food-intake, or starvation

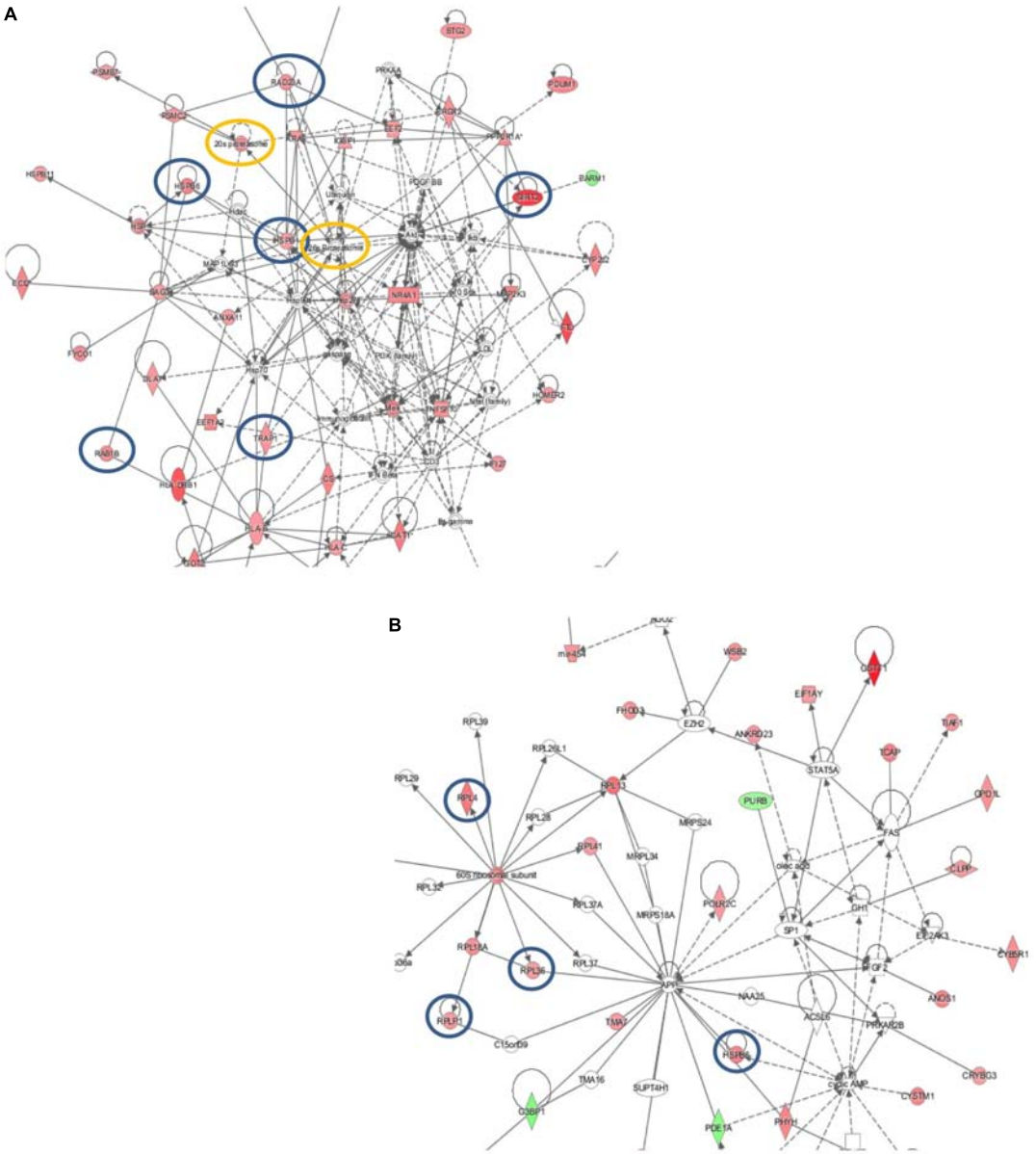

FIGURE 2 | Molecular network generated by the Ingenuity software from the differentially expressed genes in the skeletal muscle from VPG *vs* CG subjects. The main functional pathways given by Ingenuity for this molecular network are: **(A)** "Nucleic Acid, amino acid, and protein Metabolism." Highlights in this network are the RAD23A, HSPB6, HSB1, Rab1B, TRAP1, SIRT2 genes that are all correlated with increase in auto-lysosomal and proteasome-mediated protein degradation machinery and maintenance of protein quality control. **(B)** "Organ Morphology; Nervous System Development; and Function." Highlights in this network are ribosomal proteins: RPLP1, RPL4, RPL36, MRLP37 that are involved in cellular growth and proliferation pathways. In red up-regulated and in green down-regulated messenger expression is shown.

(Fulco et al., 2003). Over-expression of SIRT2 has been described in endothelial cells associated with the prevention of injuries from high-glucose and improvement of LDL metabolism in mouse models (Zhang W. et al., 2018; Zhang B. et al., 2018). There is growing evidence to indicate, that the expression of Sirtuins 1 and 2 is regulated by exercise in human and animal models, depending on type and duration (Suwa and Sakuma, 2013). However, the observations refer only to short-term bout exercise and no consensus on the topic is established so far. This is the first report investigating the expression of Sirtuins in lifelong trained subjects. We found up-regulation of SIRT2 expression in muscle from VPG subjects associated with improvement of oxidative metabolism, cardiorespiratory capacity and increased expression of key markers correlated to healthy longevity (Krustrup et al., 2010; Mancini et al., 2017).

Protein macromolecules are repeatedly exposed to potential damaging agents during their lifetime, which can cause the loss of molecular function and exhaustion of cell populations.

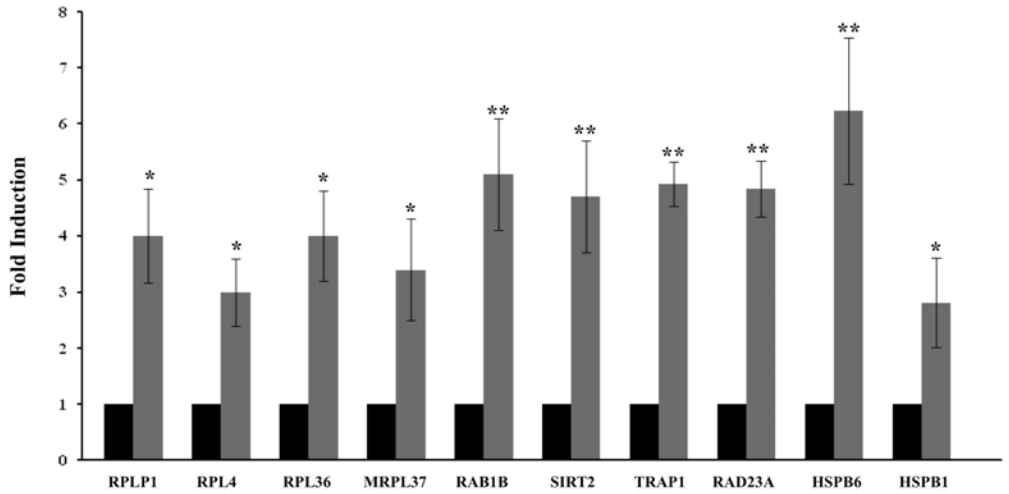

FIGURE 3 | Quantitative expression analysis of representative messengers evidenced by IPA analysis in the skeletal muscle from CG and VPG subjects. Quantitative analysis expression (RT*q*PCR) of RPLP1, RPL4, RPL36, MRPL37, RAB1B, SIRT2, TRAP1, RAD23A, HSPB6, and HSPB1 messenger expression was determined in skeletal muscle biopsies from 15 CG (black bars) to15 VPG subjects (gray bars). An arbitrary value of 1 was assigned to the expression of each messenger in CG. Data represent the means (±SEM) of three different experiments; data were compared using one-way ANOVA and differences were considered significant at $^{*}p < 0.05$ and $^{**}p < 0.01$ compared to CG.

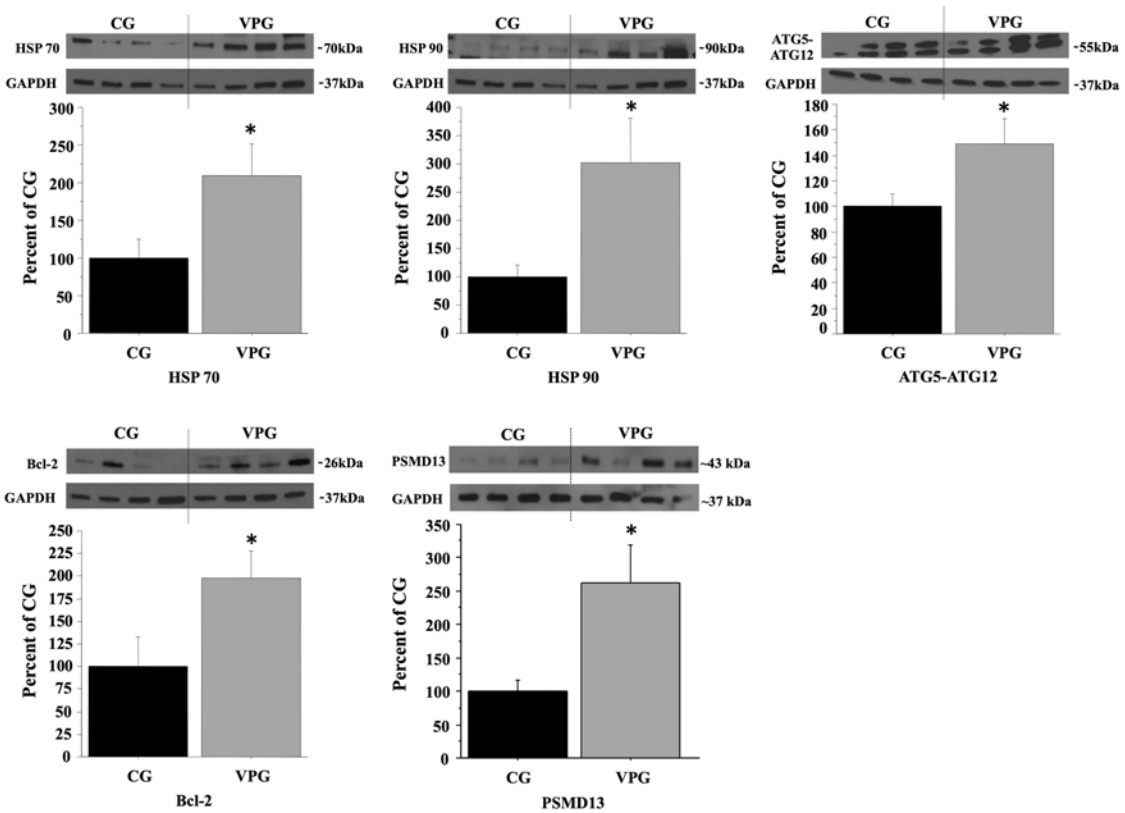

FIGURE 4 | Effects of lifelong football training on the expression of key markers involved in autophagy. Protein expression levels of HSP70, HSP90, and ATG5-ATG12 complex, involved in autophagy process, of anti-apoptotic Bcl-2, and of PSMD13 subunit of the 19S regulator complex were analyzed by Western Blotting in muscle biopsies from 15 controls (CG, black bars) to15 veterans subjects (VPG, gray bars). GAPDH served as loading control. Representative blots are reported for each protein analyzed. Data were expressed as percentage of CG expression. Comparison between groups was determined by ANOVA and represent the means (±SEM) of three different experiments. Differences were considered significant at $^{*}p < 0.05$ *vs* CG.

The induction of HSPs is a very important homeostatic adaptation favoring longevity and shielding from damage to intracellular proteins. In addition, HSPs are molecular chaperones which are activated and accumulate in skeletal muscle cells in response to exercise stress-induction (Koh and Escobedo, 2004; Paulsen et al., 2009). In the present study, expression of HSPs was higher in skeletal muscle from elderly trained subjects compared to untrained. This is likely to be a consequence of type, volume and different composition in fiber-type of skeletal muscle, as the presence of oxidative fibers is associated to higher expression of HSPs proteins (Sandri, 2013; Kim et al., 2015). Muscle contraction leads to increased levels of NAD+, AMP, and ROS in the myocytes, which in turn activates downstream signaling effectors as AMPK, p38 kinase, CaMK, Sirtuins (Vainshtein and Hood, 2016). The activation of AMPK by exercise is also requested for the activation of autophagy pathway via Bcl-2 activation (He et al., 2012). The activation of autophagy processes promotes health-metabolic effects and longevity in many tissues including skeletal muscle (Rubinsztein et al., 2011; Madeo et al., 2015; Martinez-Lopez et al., 2015). In association with the activation of MAP-kinase by exercise, PGC1-α up-regulation and suppression of mTOR expression are other key signals associated with the activation of autophagy processes (Vainshtein and Hood, 2016). We previously demonstrated that lifelong football training induces the expression of AMPK, ERK1,2 and p38kinase proteins in muscle from VPG compared to elderly untrained subjects (Mancini et al., 2017). Here, we report the up-regulation of HSPB6, HSPB1, HSP70, and HSP90 subunits, usually induced when cells are exposed to protein damaging agents, in muscle from VPG subjects. HSP70 and HSP90 mediate the check-up of the protein quality control and help cells to avoid damage-dependent apoptosis; in particular, HSP90 subunit is the key component in the assembly of the lysosomal-membrane protein type 2A complex (LAMP-2A), involved in the chaperone-mediated autophagy pathway (Salminen and Kaarniranta, 2009). Also, we found increased in the expression of ATG5-ATG12 complex and Bcl-2 protein, both positively related to authophagy pathway. In particular, the ATG5-ATG12 complex is essential for autophagosome assembly (Cuervo, 2008; Walczak and Martens, 2013) and the anti-apoptotic Bcl-2 protein plays important roles in the crosstalk between autophagy and apoptosis, being able to cooperate with ATG5 to committee the muscle cells toward autophagy if up-regulated, or toward apoptosis if down-regulated (Zhou et al., 2011). Interestingly, we also found that the expression of HSPs, Bcl-2, and ATG5-ATG12 complexes parallels the up-regulation of AMPK, PGC1-α in muscle from VPG, as previously demonstrated (Mancini et al., 2017).

Finally, we found up-regulation of RAD23A messenger expression in muscle from VPG subjects; RAD proteins are involved in the Nucleic Excision Repair process (Shuck et al., 2008), in Ubiquitin-dependent protein degradation pathway (Yokoi and Hanaoka, 2017) and in the interaction with proteasome complex finalized to delivery of ubiquitinated proteins (Liang et al., 2014). We also found the overexpression of the PSMD13 protein, named also Rpn9, a non-ATPase subunit of the 19S regulator complex, which is involved in the assembly and/or stability of the 26S proteasome (Takeuchi et al., 1999).

All these observations strongly support the hypothesis that lifelong football training enhances autophagy and in general a more efficient protein quality control processes avoiding damaged protein accumulation in skeletal muscle of elderly subjects, in turn promoting longevity.

CONCLUSION

We demonstrated that lifelong football training is able to induce transcriptional activation of key markers involved in pathways of protein quality control, in particular autophagy, and associated to improvement of intermediate metabolism and cardiovascular capacity in elderly VPG compared to untrained subjects. There is growing evidence to indicate that the expression of HSPs and autophagy process are activated by acute or chronic exercise. Increased levels of molecular chaperones and autophagy process play an important role in preventing protein damage during aging and are associated with longevity, but the molecular mechanisms leading to these effects have not been completely elucidated so far. Moreover, no consensus has been reached on exercise-type, intensity, or volume regarding the activation of this process, the effects on improvement of intermediate metabolism and longevity promotion. In this scenario, this was the first attempt to evaluate the effects mediated by lifelong football training on the autophagy and healthy aging pathway. Future studies should aim to include larger sample sizes and test the effects of post-transcriptional and epigenetic factors that contribute to the autophagy process activation mediated by football training.

AUTHOR CONTRIBUTIONS

AmM and PB conceived the manuscript, and made contributions on acquisition, analysis, and interpretation of data. MR, MH, TA, JS and PK enrolled the subjects and collected the muscle samples. SO performed bioinformatic analysis. DV, EI, and AlM carried out the experiments and performed statistical analysis of data.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphys.2019.00132/full#supplementary-material

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Long-Term Aerobic Exercise Improves Vascular Function Into Old Age: A Systematic Review, Meta-Analysis and Meta Regression of Observational and Interventional Studies

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There is an emerging body of literature relating to the effectiveness of frequent aerobic exercise as a prophylactic for age-associated dysfunction of large arteries, yet systematic evaluation and precise estimate of this effect is unknown. We conducted a systematic review and meta-analysis of controlled studies examining flow mediated dilatation (FMD) of athletic older persons and otherwise healthy sedentary counterparts to (i) compare FMD as a determinant of endothelial function between athletes and sedentary individuals and, (ii) summarize the effect of exercise training on FMD in studies of sedentary aging persons. Studies were identified from systematic search of major electronic databases from inception to January 2018. Study quality was assessed before conducting a random effects meta-analysis to calculate a pooled ES (mean difference) with 95% CI's. Thirteen studies [4 interventional (n = 125); 10 cross-sectional [including one study from the interventional analysis; (n = 485)] with age ranges from 62 to 75 years underwent quantitative pooling of data. The majority of study participants were male. Older athletes had more favorable FMD compared with sedentary controls (2.1%; CI: 1.4, 2.8%; $P < 0.001$). There was no significant improvement in the vascular function of sedentary cohorts following a period of exercise training (0.7%; CI: −0.675, 2.09%; $P = 0.316$). However, there was a significant increase in baseline diameter from pre to post intervention (0.1 mm; CI: 0.07, 0.13 mm; $P < 0.001$). In addition, there was no significant difference in endothelial independent vasodilation between the trained and sedentary older adults (1.57%; CI: −0.13, 3.27%; $P = 0.07$), or from pre to post exercise intervention (1.48%; CI: −1.34, 4.3%; $P = 0.3$). In conclusion, long-term aerobic exercise appears to attenuate the decline in endothelial vascular function, a benefit which is maintained during chronological aging. However, currently there is not enough evidence to suggest that exercise interventions improve vascular function in previously sedentary healthy older adults.

Keywords: vascular aging, vascular function, flow mediated dilation, healthy older adults, exercise

INTRODUCTION

Impaired vascular function as a result of aging occurs due to the coalition of environment, oxidative stress and inflammation (Donato et al., 2007; Seals et al., 2011). These factors result in reduced nitric oxide (NO) bioavailability, causing a failure of the vasculature to dilate in response to increases in shear stress during hyperaemia (Taddei et al., 2000, 2001; Virdis et al., 2010). Furthermore, vascular structure is also compromised with age as wall stiffness increases, reducing flexibility. Therefore, vascular dysfunction promotes cardiovascular disease (CVD) risk and contributes both to a reduction in health span and overall life expectancy (Roger et al., 2012). Given this premise there is an increasingly important but unmet need for interventions which aim to reduce inflammation and oxidative stress, while developing an environment conducive to vascular function (Seals et al., 2009).

Modifiable lifestyle factors, such as increased physical activity (PA) and/or exercise have been advocated to reduce vascular impairment and restore NO dependent vasodilatation, even in apparently healthy older cohorts (Taddei et al., 2000; Grace et al., 2015). Multiple lines of evidence, including both human and pre-clinical models demonstrate that those individuals who are regularly active enjoy superior vascular function, with lower levels of systemic inflammation and oxidative stress (Eskurza et al., 2004; Lesniewski et al., 2013; Seals, 2014; Grace et al., 2015). Despite this more than 1 in 4 of all adults, (The World Health Organization, 2017) and 85–90% of older adults in developed countries fail to meet the PA guidelines to maintain cardiovascular health (Sparling et al., 2015). This represents a contemporary challenge for researchers and healthcare providers to provide evidence-based strategies to improve engagement with PA, and to improve vascular function in older adults as a primary therapeutic target (The World Health Organization, 2010).

Vascular function, or specifically endothelial function, is commonly assessed non-invasively using the flow mediated dilation (FMD) technique. As cardiovascular events can be independently predicted by endothelial compliance, FMD has emerged as a conventional method to determine vascular function (Inaba et al., 2010). Although assessment of whole vascular function includes measures of arterial stiffness, FMD is specific to measuring endothelial function whereby endothelial NO contributes to the vasodilation of vessels after a temporary occlusion of blood flow (Green et al., 2014). However, there are few systematic interrogations of literature examining vascular function of healthy older adults, and those that have been performed, while well-executed, have key limitations. For example, Ashor et al. (2015) and Early et al. (2017) reported that exercise training improved vascular function in healthy and diseased cohorts, but in both cases, data pooling mixed young and old participants, preventing direct assessment of the effect of exercise on vascular function exclusively in older individuals. Moreover, since disease may superimpose additional vascular dysfunction on top of aging alone, it is unclear whether exercise improves vascular function due to direct effects of disease, on age *per se*, or a combination of the two. A further review by Montero et al. (2014) identified that exercise trained adults had displayed superior vascular function compared to their untrained counterparts. However, this review compared trained and untrained adults and did not address the effects of training programmes on vascular function in older adults. Moreover, their inclusion criteria encompassed studies of both middle and old age cohorts. Given that the beneficial effects of exercise may reduce with increasing age, it is difficult to interpret the results of Montero et al. (2014) in an exclusively older population.

Consequently, no meta-analysis has assessed the degree to which older (>60 years) trained individuals may have greater indices of vascular function than their untrained counterparts. Equally, there are no meta-analyses assessing the effectiveness of exercise or PA interventions in improving vascular function in similarly aged, but otherwise healthy adults. Unpicking the relationships between vascular function, aging, and exercise is necessary to enable evidence-based proposals to support health in old age. Therefore, given these gaps in the literature, the aim of this systematic review and meta-analysis was to address the following questions:

(i) Do longer-term trained older persons have more favorable vascular function, as determined by FMD, than age matched sedentary controls?
(ii) Do short-term exercise training interventions improve vascular function in previously sedentary but healthy older individuals?

METHODS

The current systematic review and Meta-analysis was conducted in accordance with the 2009 Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) checklist, and the 2000 Meta-analysis of Observational Studies in Epidemiology checklist (Stroup et al., 2000; Moher et al., 2009).

Search Strategy

An electronic database search was conducted to identify relevant exercise studies using FMD to determine vascular function of older healthy exercising and sedentary adults. PubMed/MEDLINE (abstract/title), Web of Science (title only) and ScienceDirect (abstract/title/keywords) online databases were searched, and all studies from inception to the date searched (January 2018) were included. The search string included (brachial artery flow mediated dila* OR vasodilation OR vascular function OR vascular reactivity OR vascular health OR endothelial function OR brachial artery) AND (exercise OR train* OR physical activity OR untrain* OR fitness OR program*). Filters were applied to ensure that only records in English with human participants were included in the search results. Reference lists from eligible articles were reviewed to search for additional relevant studies which may not have been present during the database search, before being subsequently screened for potential inclusion into the meta-analysis.

Inclusion and Exclusion Criteria

The inclusion criteria consisted of: (1) non-pharmacological studies of male and female human participants, (2) aged 60 years

and over, and (3) employing either cross-sectional, cohort, or randomized control trial (RCT) study designs. (4) Cross sectional design studies had to have an aged matched control group, while in the aerobic exercise intervention, studies both pre and post intervention (cohort) or RCT designs were included. (5) Studies had to include physically healthy cohorts comprising of sedentary individuals for intervention studies, or regular exercisers and sedentary individuals for cross-sectional studies. (6) Vascular function was determined using endothelium dependent FMD of the brachial artery (BA) using valid ultrasonic techniques and occlusion of the lower arm. (7) Studies must have also been published in English language literature. FMD was used as the main measure of vascular function as it is the most widely used non-invasive assessment of endothelial function, and thus gives an accurate representation of endothelial health. As FMD can predict vascular events within asymptomatic persons, it can identify impairments in vascular function within healthy older adults. There was no limitation imposed on the method of subsequent analysis, thus studies using either fixed post-deflation time points or continuous edge detection methods were included. Other measures of vascular function such as pulse wave velocity (PWV) were not included within the meta-analysis as we were specifically interested at measuring endothelial function, rather than arterial stiffness. However, since arterial diameter has been suggested as a potential confounder when assessing FMD (Atkinson and Batterham, 2015) we included analysis of this structural measure. There were also no limitations regarding the length, duration, or intensity of exercise interventions.

Studies were excluded if they (1) used pharmacological stimulus, (2) assessed a single acute exercise bout, (3) assessed resistance interventions only, or (4) assessed vascular function using a method other than FMD. In addition, (5) studies which occluded the upper arm during the FMD protocol were also excluded as this can cause a greater vasodilatory response after ischemia, possibly from mechanisms other than NO (Berry et al., 2000). As the current study aimed to assess the function of the vascular endothelium as a measure of vascular function, only lower arm occlusion was included as the post occlusion vasodilation is mainly NO mediated, and more representative of endothelium function (Doshi et al., 2001; Green et al., 2011). For example, there is evidence that increases in arterial diameter as a result of hyperaemic shear are abolished in the presence of a selective blockage of NO production within lower arm occlusion, but not upper arm occlusion (Doshi et al., 2001). We therefore excluded studies which occluded the upper arm during the FMD protocol as this method causes a greater ischemic response which may not be exclusively due to NO, and thus endothelial function. Furthermore, including lower arm occlusion also helped to standardize the FMD protocol between the included studies.

Study Selection

The literature search and selection of studies was performed by authors AC and AB. Following an initial screen of titles and abstracts (AC), full scrutiny of potentially eligible studies were independently screened by AC and AB using the specific inclusion criteria. NS arbitrated any disagreements in study inclusion.

Data Extraction

Data from the final list of eligible studies were extracted and entered into a spreadsheet (Microsoft Excel 2010). Extracted data included the following for all participant groups in each study: (1) participant ages, (2) participant activity status, (3) participant maximum oxygen uptake ($\dot{V}O_{2max}$), (4) sample size, (5) study type, (6) intervention type, frequency, duration and intensity (for interventional studies), (7) relative BA FMD percentage change (ΔFMD%), (8) BA baseline diameter (mm, when reported), (9) endothelial independent vasodilation (EIDV) (%, when measured), (10) supervised and non-supervised interventions, (11) shear rate/stress (when measured), and (12) details of FMD protocols. When studies reported both cross-sectional and interventional data from their analysis, each were screened individually to determine their eligibility. Given the suggestions of morphological adaptations in response to training (Green et al., 2012), we also sought to examine structural changes. Arterial diameter was extracted from studies to determine whether structural adaptations also occurred as a result of exercise. As arterial hyperaemic response is influenced by baseline diameter, extracting arterial diameter may help to understand why some studies show an increase in FMD or not.

ΔFMD% data were extracted as the main outcome variable. If not reported, ΔFMD% was calculated as: [(*post occlusive peak BA diameter* − *baseline BA diameter*)/ (*baseline BA diameter*)*100], where post-occlusive diameter was the peak artery diameter which occurred following cuff deflation, and baseline was the diameter determined at rest. All data were entered as mean ± standard deviation (SD). When studies reported standard error of the mean (SEM), conversion to SD was performed using the equation $SD = SEM^* \sqrt{(N)}$, where N was the number of participants. Authors of several eligible studies were contacted by email when data were not available from the text, figures, or tables. Where authors failed to respond, mean and standard deviation were extracted from graphs using the calibrated measuring function within the software "ImageJ" (Image Processing and Analysis in Java, Maryland, USA) (Abramoff et al., 2004).

Study Quality Assessment

Appraisal of study quality was undertaken using assessment tools established by the National Heart, Lung and Blood Institute (NHLBI, Bethesta, MD). Individual quality assessment tools specific to the RCT, (Quality Assessment of Controlled Intervention Studies, 2014) cohort, (Quality Assessment Tool for Before-After (Pre-Post) Studies With No Control Group, 2014) and cross-sectional study designs (Quality Assessment of Controlled Intervention Studies, 2014) were used and subsequently classified as good, fair, or poor.

Statistical Analysis

All study data were analyzed using the Comprehensive Meta-Analysis software (Biostat: V 2.2.064, Englewood, NJ, USA). Data were entered in accordance with the research questions: (1) ΔFMD% of sedentary participants compared with those who were long-term trained, and (2) pre and post Δ FMD%

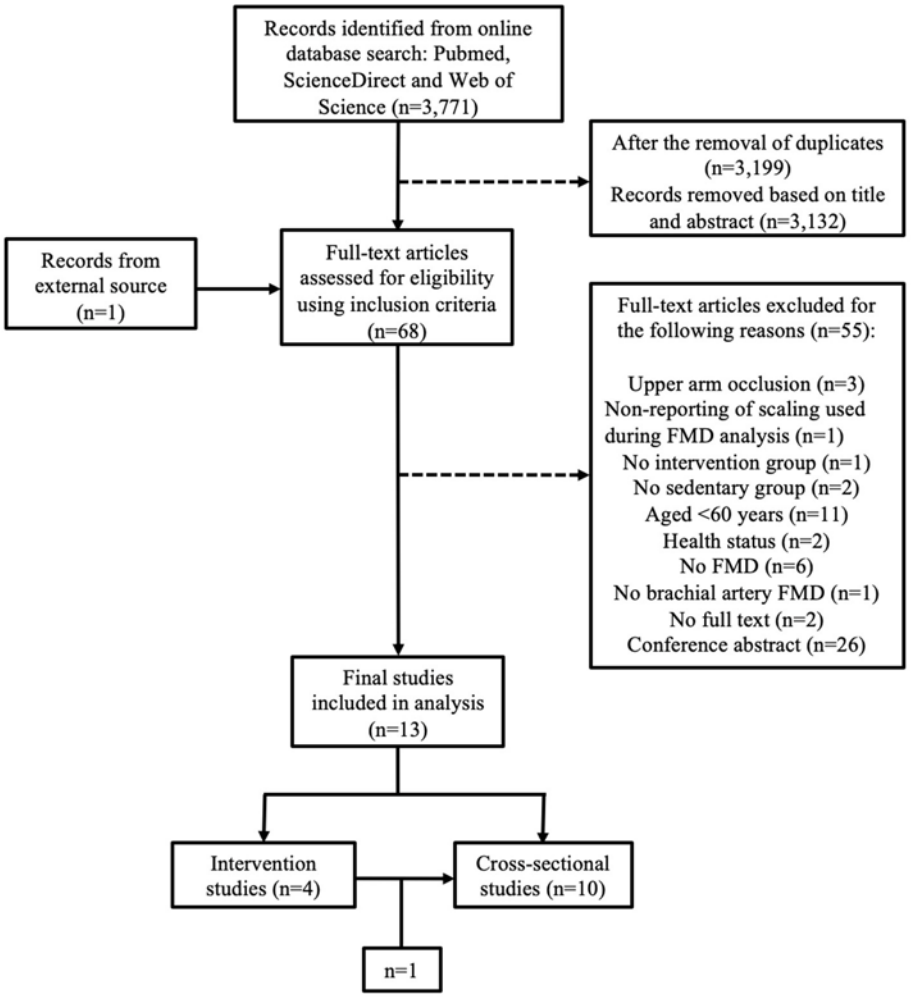

FIGURE 1 | PRISMA flow chart of study selection from the original search on Pubmed, Web of Science, and ScienceDirect.

of previously sedentary participants who had completed an exercise intervention.

The meta-analysis calculated the mean difference (MD) of BA ΔFMD%, BA diameter (mm) and BA EIDV (%), between the long-term trained vs. sedentary participants (question 1) and pre to post intervention in sedentary participants (question 2). A meta-analysis comparing the ΔFMD% of supervised and non-supervised interventions was also included within the analysis. Pooled data were analyzed using a random effects model, and differences in means in a positive direction represented an increase in FMD, baseline diameter and EIDV in favor of exercise, whereas a negative direction indicated a decrease. Between study heterogeneity was calculated for each study question, and reported as Cochran's Q and I^2, with variability of measurement characterized as low, medium, or high as 25, 50, and 75%, respectively (Higgins et al., 2003). A method of moments mixed effects meta-regression was conducted to determine if age significantly moderated the effect on ΔFMD%. Publication bias was assessed by Egger's regression. All data are presented as mean ± SD, and a P-value of ≤ 0.05 identified statistical significance.

RESULTS

Study Selection

Following the literature search from all three databases, 3,199 records were identified after the removal of duplicates. Based on title and abstract, 3,132 records were excluded, primarily due to the inclusion of participant morbidity. With the inclusion of 1 study from an external source following reference list examination, full texts of the remaining 68 articles were screened in accordance with the study inclusion and exclusion criteria. Fifty-five studies were excluded for the following reasons: non-reporting of scaling used in FMD analysis ($n = 1$); occlusion of the upper arm during the FMD protocol ($n = 3$); no intervention group ($n = 1$); no sedentary group ($n = 2$); participants were under 60 years of age ($n = 11$); participants were unhealthy ($n = 2$); FMD was not used ($n = 6$); BA FMD was not performed

TABLE 1 | Cross-sectional studies characteristics.

Study	Training status	*N*	Age (years)	% Male	Physical activity levels	$\dot{V}O_{2max}$ (ml.kg.min^{-1})	FMD % change	Overall findings	Study Quality
DeVan et al., 2013	Trained	23	62 ± 4	91	>45 min day^{-1}, ≥5 days week^{-1} for previous 2 years	($\dot{V}O_{2max}$): 42 ± 9.6	6.4 ± 1.7	↑ Trained	Fair
	Sedentary	35	62 ± 5	86	<30 min day^{-1}, ≤2 days per week for previous 2 years.	($\dot{V}O_{2max}$): 31 ± 5.9	5.3 ± 2.2		
Eskurza et al., 2005	Trained	12	66 ± 4	100	>3 sessions week^{-1} of vigorous endurance exercise >2 years	41.3 ± 4.2	5.9 ± 1.7	↑ Trained	Good
	Sedentary	9	62 ± 6	100	No regular PA >2 years.	29.1 ± 6.3	3.9 ± 2.1		
Franzoni et al., 2005	Trained	16	64 ± 6	100	$\dot{V}O_{2max}$ >50 ml.kg.min^{-1}. Vigorous endurance exercise >5 times week^{-1} and participate in national and international road-running races. Training for 37 ± 5 years.	54.7 ± 3.7	5.3 ± 3.2	↑ Trained	Good
	Sedentary	16	64 ± 4	100	$\dot{V}O_{2max}$ <45 ml.kg.min^{-1}	28 ± 5.9	2.3 ± 1		
Galetta et al., 2006	Trained	30	65 ± 5	100	$\dot{V}O_{2max}$ >40 ml.kg.min^{-1} Competitive endurance runners since 40 years. 1–2 h day^{-1} for 5 days (3 days long distance running and 2 days walk-weight training) or 5–10 km weekly or 20 km once every 2 weeks.	45.7 ± 3.7	6.2 ± 2	↑ Trained	Good
	Sedentary	28	66 ± 6	100	$\dot{V}O_{2max}$ <35 ml.kg.min^{-1} and no regular exercise	28 ± 5.9	2.4 ± 1.5		
Grace et al., 2015	Trained	17	61 ± 5	100	Life-long exercisers and completed on average 280 min exercise training week^{-1}. Most participants were actively competing in endurance sports.	39.2 ± 5.6	5.4 ± 1.4	↑ Trained	Good
	Sedentary	22	63 ± 5	100	No formal exercise programme for ≥30 years.	27.2 ± 5.2	3.4 ± 1.5		
Jensen-Urstad et al., 1999	Trained	9	75 ± 3	100	Participants had been and were still among the best in their respective age groups in running since ages of 15–25. Between 3–7 h strenuous exercise week^{-1}.	41 ± 7	4.8 ± 5	↑ Trained	Good
	Sedentary	11	75 ± 2	100	Sedentary or moderately active.	27 ± 5	1.1 ± 2.1		
Pierce et al., 2011a	Trained	13	62 ± 7	100	Vigorous aerobic exercise (competitive running, cycling and triathlons) ≥ 5 days week^{-1} for ≥ 45 min day^{-1} >5 years.	42 ± 3.6	6.3 ± 1.8	↑ Trained	Good
	Sedentary	28	63 ± 5	100	No regular aerobic exercise (<30 min day^{-1}, <2 days week^{-1}, ≥2 years).	29 ± 5.3	4.9 ± 2.1		
Pierce et al., 2011b	Trained	65	62 ± 6	69	Vigorous aerobic exercise (competitive running, cycling and triathlons) >5 days week^{-1} for >45 min day^{-1} >5 years.	41.5 ± 7.7	6.1 ± 2.9	↑ Trained	Good
	Sedentary	102	62 ± 10	59	No regular aerobic exercise (<30 min day^{-1}, <2 days week^{-1}, >2 years).	27.4 ± 6.6	4.8 ± 2.3		
Walker et al., 2009	Trained	16	66 ± 4	100	>3 sessions week^{-1} vigorous aerobic endurance exercise.	42.8 ± 5.2	6.2 ± 2.6	→	Good
	Sedentary	15	66 ± 4	100	No regular exercise for 2 years	29.9 ± 4.7	4.8 ± 1.6		
Eskurza et al., 2004	Trained	9	64 ± 6	100	>3 sessions week^{-1} vigorous aerobic endurance exercise for ≥ 2 years	40 ± 6	7 ± 1.8	↑ Trained	Good
	Sedentary	9	64 ± 6	100	Sedentary (No regular PA) for ≥ 2 years	32 ± 3	4.6 ± 0.6		

Study characteristics of cross-sectional studies within question 1 [↑ identifies that a significant increase in FMD% change occurred ($P \leq 0.05$) and → identifies no change ($P > 0.05$)]. N, number of participants; % Male, percentage of male participants: $\dot{V}O_{2max}$, maximum aerobic capacity; FMD% change, flow mediated dilation percentage change; PA, physical activity). Values are displayed as mean ± SD.

($n = 1$); conference abstract only ($n = 26$); and no full text available ($n = 2$). Consequently, 13 studies were deemed eligible for analysis. One study consisted of both an intervention and a cross-sectional design and analysis, however only the cross-sectional section met all inclusion criteria (Pierce et al., 2011b). Additionally, one of the interventional studies contained both baseline and post intervention data and was therefore included in both comparisons (Grace et al., 2015). As a result the overall analysis contained 10 cross-sectional (Jensen-Urstad et al., 1999; Eskurza et al., 2004, 2005; Franzoni et al., 2005; Galetta et al., 2006; Walker et al., 2009; Pierce et al., 2011a,b; DeVan et al., 2013; Grace et al., 2015) and 4 interventional studies (Thijssen et al., 2007; Klonizakis et al., 2014; Suboc et al., 2014; Grace et al., 2015) (**Figure 1**). The included studies were believed to

be mainly of "good" quality as measured using the National Heart, Lung and Blood Institute study quality assessment criteria (**Tables 3–5**).

The cross-sectional and interventional analyses were found to have moderate and low heterogeneity ($I^2 = 63.6\%$; $P = 0.003$; and $I^2 = 47.4$, respectively). To compensate for the heterogeneity identified, the meta-analysis was conducted using random effect models, as recommended by the Cochrane guidelines (Higgins and Green, 2011).

Question 1: Cross-Sectional Study Analysis

The 10 cross-sectional studies included 485 participants (210 long-term trained and 275 sedentary) (Jensen-Urstad et al., 1999; Eskurza et al., 2004, 2005; Franzoni et al., 2005; Galetta et al., 2006; Walker et al., 2009; Pierce et al., 2011a,b; DeVan et al., 2013; Grace et al., 2015). Ages ranged from 62 to 75 years in the sedentary participants (mean of 65 years) and from 61 to 75 years in the long-term trained participants (mean of 65 years). Cohort sizes ranged from $n = 9$ to $n = 65$ participants in the long-term trained and $n = 9$ to $n = 102$ in the sedentary groups. Seven of the long-term trained groups and 5 of the sedentary groups contained <20 participants (Jensen-Urstad et al., 1999; Eskurza et al., 2005; Franzoni et al., 2005; Walker et al., 2009; Pierce et al., 2011a; Grace et al., 2015). Two of the 10 studies contained both male and female participants (Pierce et al., 2011b; DeVan et al., 2013). Studies with both males and females were included as it has previously been identified that post-menopausal females display a similar BA FMD compared to males of the same age (Jensen-Urstad and Johansson, 2001). Additionally, as only one study presented male and female data individually, results for each sex were analyzed together. Long-termed trained participants consisted of endurance runners, swimmers, and cyclists who trained at least three times per week and with regular exercise participation between 2 and 37 years (**Table 1**).

Studies described cuff occlusion pressures to range from 40 mmHg above systolic blood pressure to 300 mmHg and remained inflated between 4 and 5 min. ΔFMD% was analyzed from all 10 cross-sectional studies and ranged from 4.8 ± 5 to 7 ± 1.8% in the long-term trained participants and 1.1 ± 2.1 to 5.3 ± 2.2% in sedentary participants. ΔFMD% values normalized for shear stress were reported in one study (Eskurza et al., 2005), and for hyperaemic shear in one other (Eskurza et al., 2004). All 10 cross-sectional studies reported mean baseline BA diameter data, whereas only 9 studies reported EIDV data.

Data pooling from the meta-analysis indicated that ΔFMD% was significantly greater in long-term trained vs. sedentary older adults (MD: 2.1, 95% CI: 1.4, 2.8%; $P < 0.001$; **Figure 2**). Moderate heterogeneity was observed between the 10 cross-sectional studies ($I^2 = 63.6\%$; $P = 0.003$). Egger's regression determined that there was a low risk of publication bias ($P = 0.7$). The meta-regression found no significant effect of age on ΔFMD% ($P = 0.08$; **Table 6**). Data pooling identified that EIDV was not significantly different between the trained and untrained participants (MD: 1.57; 95% CI: −0.132, 3.274%; $P = 0.07$; **Figure 4**), and baseline diameter of the BA was also similar between the two groups (MD: −0.1 mm; 95% CI: −0.09 mm, 0.29 mm; $P = 0.30$; **Figure 3**).

Question 2: Intervention Study Analysis

The 4 intervention studies consisted of 3 cohort designs (Thijssen et al., 2007; Klonizakis et al., 2014; Grace et al., 2015) and one RCT (Suboc et al., 2014). Included in the 4 studies were 125 sedentary participants. Sample sizes ranged from 8 to 77 participants, where two of the studies contained <20 participants (Thijssen et al., 2007; Klonizakis et al., 2014). The ages of participants ranged from 62 ± 7 to 70 ± 1 years, with a mean age of 65 years. One of the studies contained both male and female participants (Suboc et al., 2014), and one study recruited only postmenopausal females (Klonizakis et al., 2014). Again, as the study containing both males and females did not present their results separately, data for each sex was analyzed together (**Table 2**).

Cuff inflation during the FMD protocol ranged from 50 mmHg above systolic blood pressure to approximately 220 mmHg for a 5 min period. The duration of study interventions lasted between approximately 9 to 84 sessions from 2 to 12 weeks, with 3 of the studies including exercise interventions of a moderate to high intensity. One of the cohort studies included two separate interventions—one consisting of high intensity, and the other a moderate intensity intervention (Klonizakis et al., 2014). Frequency of interventions ranged from once every 5 days to 7 days per week and lasting between 20 min to 1 h per day.

For the cohort studies ΔFMD% was calculated from baseline and post measures, while post intervention and control values were analyzed in the RCT study. ΔFMD% ranged from 3.4 ± 1.5 to 8.9 ± 7.9% pre-intervention to 5.4 ± 1.4 and 7 ± 4.3% post intervention. All four of the studies reported baseline BA diameter data, whilst only two studies reported data for EIDV.

The meta-analysis suggests that there was no significant improvement in ΔFMD% after the exercise interventions in previously sedentary older adults (MD: 0.707, 95% CI: −0.68, 2.1%; $P = 0.316$; **Figure 2**). Heterogeneity of the 5 intervention studies were calculated as low ($I^2 = 47.4\%$, $P = 0.107$, and Egger's regression determined that some publication bias may be present ($P = 0.047$). The meta-analysis identified that there was no increase in EIDV from pre to post intervention (MD: 1.48; 95% CI: −1.3, 4.3%; $P = 0.303$; **Figure 4**), however there was a significant increase in BA baseline diameter post intervention (MD: 0.1 mm; 95% CI: 0.07 mm, 0.13 mm $P < 0.001$; **Figure 3**). Finally, ΔFMD% was not affected by whether the interventions were supervised or non-supervised (heterogeneity $p = 0.927$; **Figure 5**).

DISCUSSION

This systematic review and meta-analysis set out to determine the effects of short and long-term exercise training on vascular

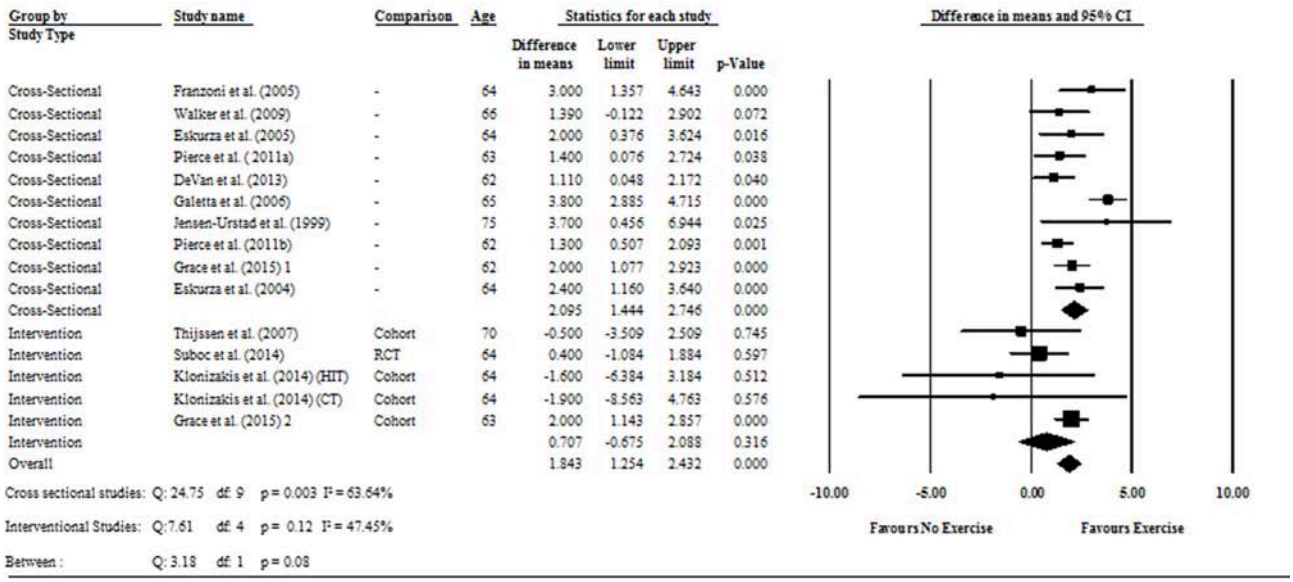

FIGURE 2 | Forest plot of the meta-analysis with mean differences of FMD percentage change between trained vs. sedentary healthy older adults (question 1), and FMD percentage change pre to post exercise intervention in previously sedentary healthy older adults (question 2). Outcomes of questions 1, 2, and the moderator analysis are also presented.

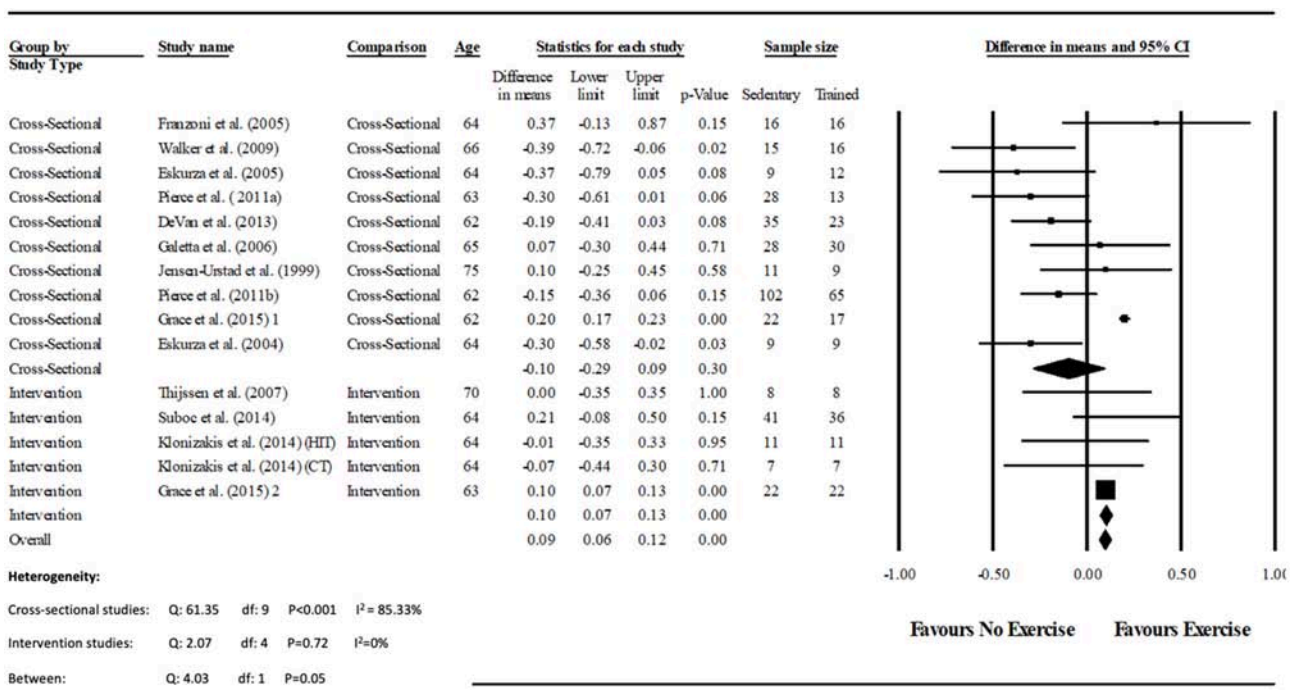

FIGURE 3 | Forest plot of the meta-analysis of brachial artery baseline diameter (mm) between trained vs. sedentary healthy older adults, and pre to post exercise intervention in previously sedentary healthy older adults. The heterogeneity and moderator analysis are also presented.

TABLE 2 | Interventional study characteristics.

Study	Study design	N	Age (years)	% Male	Exercise intervention				Study duration	$\dot{V}O_{2max}$ (ml.kg. min^{-1})	FMD % change	Overall findings	Study Quality
					Exercise type	Intensity	Session duration	Frequency					
Thijssen et al., 2007	Cohort	8	70 ± 1	100	Cycling training on an ergometer	65% HRR and gradually increasing by 5% until 85%	20 min	3 days week^{-1}	8 weeks	Pre: 30.8 ± 4.8 Post: 33.3 ± 5.5	Pre: 6.9 ± 3.4 Post: 6.4 ± 2.7	→ FMD%	Good
Suboc et al., 2014	RCT	77	PED: 64 ± 7	PED: 61	PED ($n = 36$) walking CON ($n = 41$)	Increase PA by 10% weekly above baseline to reach an average of 10,000 steps day^{-1}	–	Daily	12 weeks	–	Post: CON:6.3 ± 2.7	→ FMD%	Good
			CON: 62 ± 7	CON: 76							HIT:6.7 ± 3.9		
Klonizakis et al., 2014	Cohort	18	HIT: 64 ± 7	0	HIT ($n = 11$): cycling intervals on ergometer	HIT: 100% PP and light active recovery intervals at 30 W	HIIT: 10 × 1 min intervals with 1 min recovery between each	3 times week^{-1}	2 weeks	HIT; Pre: 20.4 ± 3.4 Post: 22.6 ± 3.1	HIT; Pre:8.1 ± 7.2 Post:6.5 ± 3.7	→ FMD%	Good
			CT: 64 ± 4		CT ($n = 7$): continuous cycling	CT: 65% PP	CT: 40 min			CT; Pre: 25 ± 7.4 Post: 26.7 ± 5.4	CT; Pre: 8.9 ± 7.9 Post: 7 ± 4.3	→ FMD%	
Grace et al., 2015	Cohort	22	63 ± 5.2	100	Progressive conditioning exercise: ACSM guidelines	Conditioning exercise ACSM guidelines (Chodzko-Zajko et al., 2009) and 50% PP HIIT sprints	Conditioning exercise: 150 min week^{-1} ≥30 min day^{-1} (ACSM guidelines Chodzko-Zajko et al., 2009)	Conditioning: ≥5 days week^{-1} (ACSM guidelines Chodzko-Zajko et al., 2009)	Conditioning: 6 weeks	Pre: 27.2 ± 5.2	Pre: 3.4 ± 1.5	↑ FMD%	Good
					HIIT: sprints on cycle ergometer.		HIIT: 6 × 30 s sprints with 3 min break between each	HIIT: once every 5 days	HIIT: 6 weeks	Post: 32.2 ± 5.6	Post: 5.4 ± 1.4		

Study characteristics of interventional studies within question 2. ↑ identifies a significant increase in FMD% change ($P \leq 0.05$) and → identifies no significant change ($P > 0.05$). N, number of participants; % Male, percentage of male participants; $\dot{V}O_{2max}$, maximum aerobic capacity; FMD% change, flow mediated dilation percentage change; PA, physical activity; HRR, heart rate reserve; WR_{max}, maximum work rate; PP, peak power; HIT, high intensity training; CT, continuous training; ACSM, American College of Sports Medicine; HIIT, high intensity interval training; PED, pedometer; CON, control. Values are displayed as mean ± SD.

function and has 2 main findings. First, pooled data from cross-sectional studies demonstrate that long-term trained healthy older adults have superior vascular function compared with their sedentary but otherwise healthy counterparts; and second that FMD may not improve in sedentary individuals who undertake shorter-term aerobic exercise interventions although there may be an increase in arterial diameter. These data are the first pooled synthesis of controlled observational and interventional studies using healthy older cohorts. The current meta-analysis also allows some comparison between observational and interventional studies since we used the same inclusion and exclusion criteria for studies in both comparisons. Moreover, since all participants were apparently healthy and not taking any medication, the results may provide some insight into the effect of short and long-term training on aging *per se* rather than on aging in combination with comorbidities. The current study therefore differs from previous meta-analyses which combined healthy and diseased

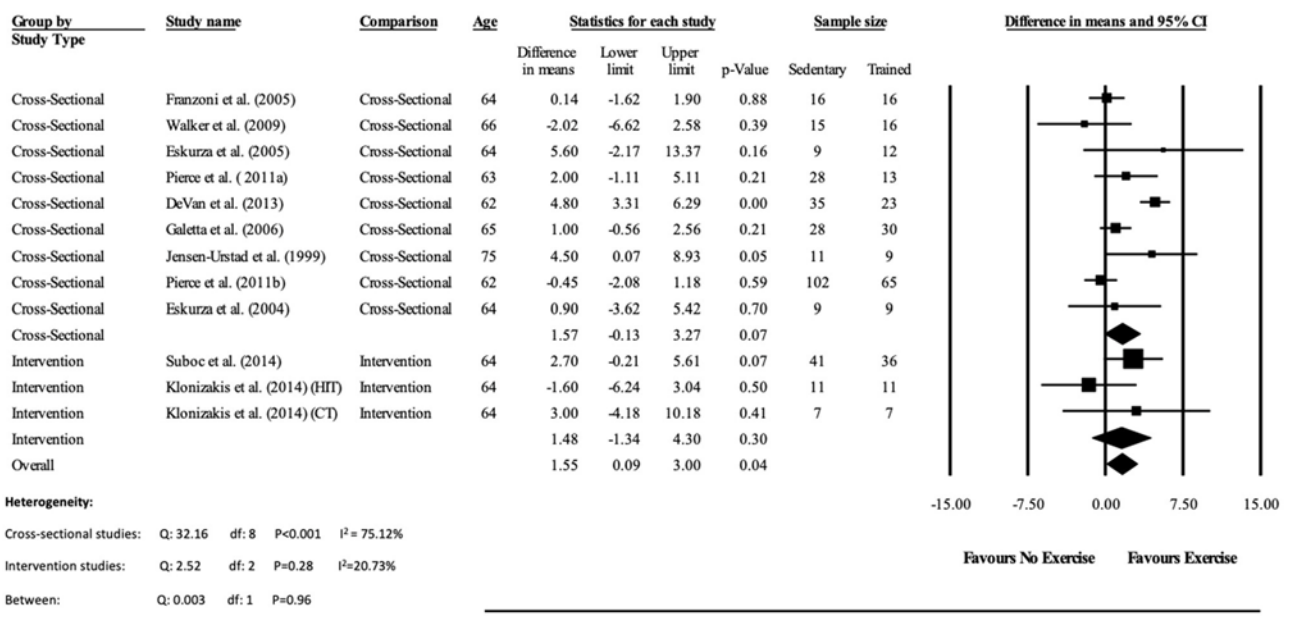

FIGURE 4 | Forest plot of the meta-analysis of endothelial independent vasodilation percentage change (EIDV%) between trained vs. sedentary healthy older adults, and pre to post exercise intervention in previously sedentary healthy older adults. The heterogeneity and moderator analysis are also presented.

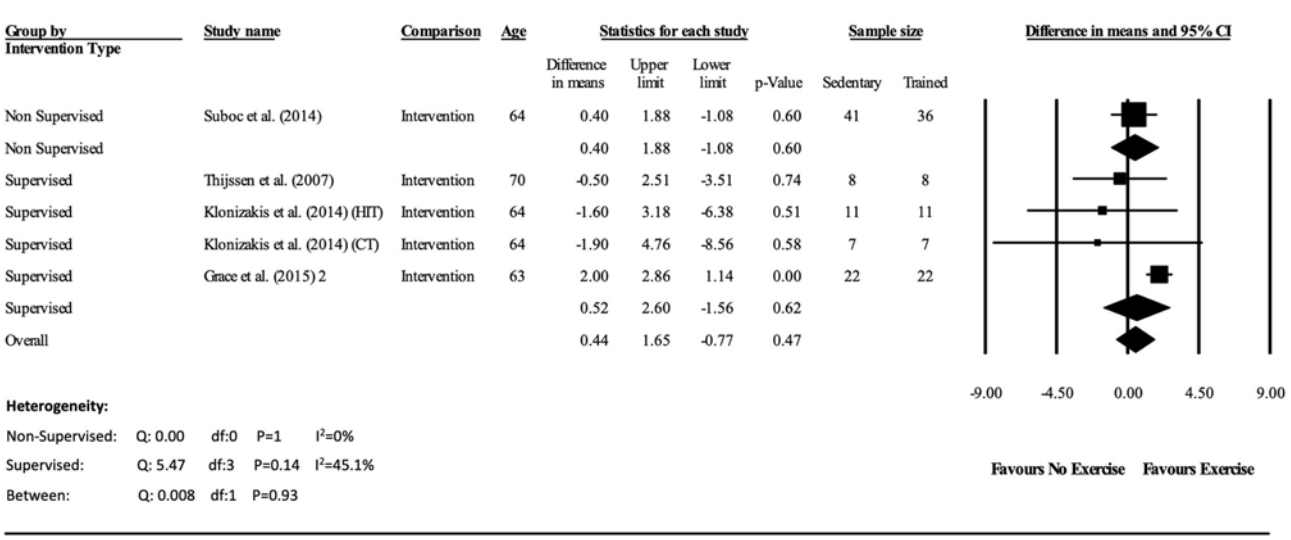

FIGURE 5 | Forest plot of the meta-analysis of brachial artery FMD percentage change within supervised and non-supervised exercise interventions. Heterogeneity and moderator analysis are also presented.

participants, or mixed middle aged and older participants together in their analysis (Montero et al., 2014; Ashor et al., 2015; Early et al., 2017). Therefore, to our knowledge the current study is the first meta-analysis to identify vascular function exclusively in healthy adults aged 60 years and over who are either endurance trained or untrained, or who have completed an endurance intervention having previously been sedentary.

Question 1—Do Long-Term Trained Older Individuals Demonstrate Superior Vascular Function, as Determined by FMD, Than Age Matched Sedentary Controls?

As outlined above, the meta-analysis indicated that endurance exercise training is associated with improved vascular function, despite considerable differences in study designs. Indeed, within these 10 studies there was a wide range in prior exercise experience, with trained cohorts ranging from a minimum of 2 years training through to life-long exercisers and which may have contributed to the heterogeneity in this comparison.

Nevertheless, the data indicates that exercise provides a protective mechanism in long-term trained participants, who experience a slower deterioration of vascular function, compared with sedentary but otherwise healthy older adults. While the protective mechanism of exercise remains to be fully elucidated it is widely believed that the hyperaemic effects of exercise, and the repeated exposure of the endothelium to bouts of increased shear stress, act to reduce the deleterious effects inflammation and oxidative stress (Tinken et al., 2010). The combination of these effects enhances the bioavailability of NO, increasing vasodilation during the hyperaemic response following an ischaemic stimulus (Taddei et al., 2000). From the data presented it appears that the vasculature of healthy older long-term trained adults may be exposed to these physiological mechanisms at a level which preserves vascular function relative to their sedentary counterparts.

Previous work has suggested that trained individuals have shown to exhibit wider peripheral artery diameters when compared to untrained individuals. Often referred to as the athletes artery, it is thought to represent arterial remodeling in response to repeated bouts of shear stress which occur during exercise. It is hypothesized that a widened vessel requires less vasodilation during periods of reactive hyperaemia, resulting in reduced dilatation during FMD (Green et al., 2012, 2013). However, the analysis of long-term trained athletes, in whom any such adaptation may be greatest fails to support this concept since arterial diameters of the trained individuals did not differ significantly from the sedentary cohort. Although allometric scaling of the data would have been beneficial, a lack of anthropometric data within the studies meant that scaling was not possible. Nevertheless, the present meta-analysis identified that the trained group had a greater vascular function compared to the untrained group, whilst both displaying a similar baseline artery diameter (**Figure 3**). These findings agree with a meta-analysis by Montero et al. (2014) who identified that masters athletes BA diameters were similar to untrained controls, whilst FMD was also significantly greater.

The meta-regression aimed to identify if increasing age reduced the improvement in FMD seen in trained individuals compared to sedentary controls. In this case the lack of a significant association indicated that the difference between the two cohorts was not reduced with advancing age Therefore, the present findings underline the notion that exercise can support vascular function, well into the eight decade (Grace et al., 2015). It is also notable, that most (Walker et al., 2009; Pierce et al., 2011a; DeVan et al., 2013), but not all (Franzoni et al., 2005), previous work has demonstrated that the vascular function of healthy young untrained adults is greater than older trained adults. These data suggest that, even in well-trained individuals, there may some unavoidable decrement in vascular function with age. In addition, the range in mean ages of the included observational studies was relatively narrow (62–75 years); future research should attempt to determine if improvements in vascular function is maintained in those who participate in long-term endurance exercise.

Question 2—Do Exercise Training Interventions Improve Vascular Function in Previously Sedentary Healthy Older Persons?

The results of observational studies support the use of exercise to ameliorate the age related decline in vascular function. However, as is the case in all observational studies, the inability to directly link the main outcome (i.e., FMD) to the exposure of interest (i.e., exercise training) limits the confidence that may be placed in conclusions from such studies (Higgins and Green, 2011). In an attempt to overcome this, the present review further assessed the effectiveness of prospective aerobic intervention studies with otherwise sedentary participants.

Data pooling indicated that the short term training programmes within the included studies (2–12 weeks) did not significantly improve vascular function. This finding is at odds with the results of the cross-sectional assessment of endurance trained participants and controls reported above. However, the training interventions were associated with a significant increase in diameter of the BA at baseline (i.e., immediately prior to cuff inflation; **Figure 3**). Since included studies where not mechanistic in nature, assessing the lack of effect is speculative. For example, it may be that in this age group the recovery of vascular function is slow and vascular remodeling (i.e., "the athletes artery") becomes a primary method of adaptation (Green et al., 2012). Similarly, it may be that since most studies used cycling exercise, early adaptation is focussed on the most active vascular beds of the lower limbs, and assessment of brachial arterial function is insufficiently sensitive (Thijssen et al., 2007).

Alternatively, there may not be any vascular dysfunction, and the lack of effect on FMD is a result of the increased baseline diameter which aids total blood flow. Indeed increased shear stress, as a result of an exercise has been suggested to cause systemic arterial remodeling (Maiorana et al., 2011). It is noteworthy that long-term training improved FMD without morphological changes (rationale 1), while short term training increased baseline diameter without improving FMD. Given the interventions were between 2 and 12 weeks, and that vascular remodeling may occur within that time frame (Tinken et al., 2008), it is possible that this is responsible for increased baseline diameter within short term interventions. However, the majority of the interventional studies only measured vascular function before and after the exercise intervention. The "pre-post" nature of FMD assessments means that it is possible that

TABLE 3 | Cross-sectional study quality.

	Research question clear?	Study population clear? (including without date and place)	Criteria same within both groups?	Sample size calculation?	Exposure measures clear, reliable and valid? Implemented consistently?	Outcome measures clear, reliable and valid? Implemented consistently?	Blinded assessment of FMD?	Follow-up after baseline 20% or less?	Key potential confounding variables considered?
DeVan et al., 2013	Y	Y	Y	N	N	N	NR	Y	Y
Eskurza et al., 2005	Y	Y	Y	N	Y	Y	Y	Y	Y
Franzoni et al., 2005	Y	Y	Y	N	Y	Y	NR	Y	Y
Galetta et al., 2006	Y	Y	Y	N	N	Y	NR	Y	Y
Grace et al., 2015	Y	Y	Y	Y	Y	Y	N	Y	Y
Jensen-Urstad et al., 1999	Y	Y	Y	N	N	Y	NR	Y	Y
Pierce et al., 2011a	Y	Y	Y	N	N	Y	Y	Y	Y
Pierce et al., 2011b	Y	Y	Y	N	Y	Y	Y	Y	Y
Walker et al., 2009	Y	Y	Y	Y	Y	Y	NR	Y	Y
Eskurza et al., 2004	Y	Y	Y	N	Y	Y	Y	Y	Y

Study quality assessment of the cross-sectional studies included in question 1 developed by the National Heart, Lung and Blood Institute (NHLBI) (Quality Assessment Tool for Observational Cohort Cross-Sectional Studies, 2014). Y, yes; N, no; NR, not reported.

improvements were missed by the time post measures where performed. Indeed, future work should assess the presence (or otherwise) of changes in vascular function throughout the time course of training interventions. However, it is also worth noting that there was no improvement in vascular function after 2 weeks of either continuous or high intensity training within the study by Klonizakis et al. (2014), suggesting that relatively fast improvements in vascular function may not always occur.

It is also worth noting several limitations of the literature pertaining to interventions in this age group. There were a relatively small number of interventional studies which met the inclusion criteria, meaning that again there was a relatively limited age range of 62–70 years. Moreover although the studies included within the meta-analysis were endurance based, the 4 included studies consisted of low, moderate and high exercise intensities, and ranged between 2 and 12 weeks, all of which may have contributed to the moderate heterogeneity of the pooled data. Future analyses may benefit from analyzing intensity zones as these may affect vascular function differently (Ashor et al., 2015; Ramos et al., 2015; Early et al., 2017), and the greatest effect in the available literature was observed following high intensity interval training (Grace et al., 2015). However, due to the limited number of studies included within the current analysis, it was not possible to categorize studies based on their intensities.

However, despite the lack of improvements in vascular function following exercise interventions, there are a number of other physiological advantages of exercise such as increased muscle strength and power (Reid and Fielding, 2012), as well as a reduction in other risk factors involved in aging (Seals, 2014; Barnes, 2015). It therefore seems prudent to advise older adults who begin exercising to remain active indefinitely in order to enjoy other health benefits associated with PA and exercise; not least as detraining may reverse potential improvements in various physiological factors (Pullin et al., 2004).

Additionally, the meta-analysis identified that EIDV did not change significantly in either the trained vs. untrained participants in the cross-sectional analysis, or pre vs. post training within the interventional analysis, however combined pooling suggested a small effect. As EIDV is commonly used as a control test to assess whether improvements in FMD are mainly NO mediated, these data suggest that improvements in trained participants FMD may be due to exercise induced improvements of the vascular endothelium, rather than alterations in vascular smooth muscle within the tunica media (Maruhashi et al., 2013). Conversely, the previously sedentary participants who completed an exercise intervention found no overall improvements in either endothelial function, or vascular smooth muscle cell function.

Study Quality

The quality of most studies was determined as "good" (see **Tables 3**–**5**); however, some limitations were noted. Firstly, the majority of both observational and cohort studies recruited only a small number of participants (<20 participants per group) which increases the chances of type 2 error, while also increasing the risk of finding a disproportionately large effect size (Button et al., 2013). Furthermore, the current meta-analysis only identified a single RCT from the literature which

TABLE 4 | Cohort study quality.

	Research question clear?	Participant eligibility criteria clear? (without date and names)	Were participants representative of general population of interest?	Were all eligible participants enrolled?	Was sample size sufficient to provide confidence in the findings?	Was the intervention clearly described and delivered consistently?	Outcome measures clear, reliable and valid? Implemented consistently?	Blinded assessment of FMD?	Loss to follow-up after baseline <20%	Did the statistical method examine changes pre and post intervention?
Grace et al., 2015	Y	Y	Y	Y	Y	Y	Y	NR	Y	Y
Klonizakis et al., 2014	Y	Y	Y	Y	CD	Y	Y	NR	Y	Y
Thijssen et al., 2007	Y	Y	Y	Y	Y	Y	Y	NR	Y	Y

Study quality assessment of the cohort studies included in question 2 developed by the National Heart, Lung and Blood Institute (NHLBI) (Quality Assessment Tool for Before-After (Pre-Post) Studies With No Control Group, 2014). Y, yes; N, no; NR, not reported; CD, cannot determine.

met the study's inclusion criteria. Given that RCTs are widely considered the most internally valid design to determine cause and effect (Evans, 2003), future studies should investigate the use of this approach. Furthermore, few studies reported that outcome assessors were blind to the participant's group allocation during assessment of FMD, increasing the risk of performance bias (Higgins and Green, 2011).

Moreover, while the included intervention studies performed outcome assessments at the cessation of the training programme, no studies included a sufficient follow-up to allow for determination of the longevity of any beneficial effect. Although FMD is a useful predictor of vascular endothelial function, longer follow-up periods would be useful to identify whether improvements in FMD from exercise translates into a decreased incidence of vascular disease and mortality. Additionally, there is also the need for more comprehensive reporting of participant characteristics, including confirmation of medical history, training status, training frequency and duration (e.g., mins per week), and intensity as many studies lack sufficient detail.

Strengths and Limitations

This is the first systematic review and meta- analysis to focus on the effects of exercise on vascular function via FMD in healthy older adults using both cross-sectional and interventional studies; however, a number of limitations should be noted. Firstly, although FMD measurement protocols were similar between studies, some minor differences in protocols were evident. Studies measured post occlusion BA diameter for different durations, ranging from 90 s to 10 min after cuff deflation. In addition to differences in methodology, there were also a wide range of baseline ΔFMD% values in intervention studies (3.4 ± 1.5 to 8.9 ± 4.9%). Furthermore, only a small number of studies normalized FMD values for hyperaemic stimulus (Eskurza et al., 2004, 2005). Therefore, the varied protocols and lack of FMD normalization by shear may have all contributed to the differences between the studies. Also, for the purposes of this analysis we defined vascular function as brachial artery FMD response. However, it is plausible that analysis of micro-vascular function, different regions of the vascular system, or analysis of other measures of vascular health (e.g., pulse wave analysis or pulse wave velocity) may provide different results.

Moreover, although the interventional studies were specifically aerobic in nature, the intensities of the exercises differed considerably. For example, Suboc et al. (2014) conducted a walking intervention, which was of relatively low intensity, whereas (Klonizakis et al., 2014) documented a high intensity protocol requiring participants to work at 100% of their peak power output. However, due to the lack of interventional studies meeting the inclusion criteria, all 4 studies had to be analyzed together. A greater number of interventional studies are therefore required within this specific cohort to investigate whether varying exercise intensities would influence vascular improvements differently. Additionally, within the cross-sectional studies the lack of detail, and the use of qualitative descriptors of training intensity in the trained older individuals (e.g., "vigorous exercise") makes more detailed comparison

TABLE 5 | Randomized control trial study quality.

	Described as a RCT?	Was the method of randomization adequate?	Was the treatment allocation concealed?	Were the investigators blinded?	Were groups similar at baseline on important characteristics that could affect outcomes?	Was overall drop-out rate ≤20%	Was the differential drop-out rate at endpoint ≤15%?	High adherence to intervention protocols?	Were other interventions avoided or similar in the groups?	Outcome measures clear, reliable and valid? Implemented consistently?	Sample size sufficient to detect difference with at least 80% power?	Were outcomes reported or subgroups analyzed pre-specified?	Were all randomized participants analyzed in the group they were originally assigned?
Suboc et al., 2014	Y	NR	NR	Y	Y	Y	Y	N	Y	Y	Y	Y	Y

Study quality assessment of the RCT study included in question 2 developed by the National Heart, Lung and Blood Institute (NHLBI) (Quality Assessment of Controlled Intervention Studies, 2014). RCT, randomized controlled trial; Y, yes; N, no; NR, not reported.

TABLE 6 | Meta-regression analysis.

Comparison	Covariate	n	q	df	SE	β	95% CI	*P*-value
Trained vs. sedentary	Age	10	3.17	1	0.12	0.22	0.02, 0.46%	0.08

Meta-regressions assessing the effect of age on FMD percentage change.

of the effects of long-term training in this cohort difficult. Furthermore, although there were no differences in ΔFMD% between supervised and non-supervised interventions, only one non-supervised intervention was included in the meta-analysis. Therefore, until further non-supervised interventional protocols meeting the inclusion criteria become available, this result should be interpreted with caution.

All of the studies included within the meta-analysis assessed vascular function of the BA via FMD, despite the interventions and longer-term exercise routines consisting primarily of lower-limb exercise. However, it has previously been identified that cycling can significantly improve vascular function of the non-exercising upper limbs (Birk et al., 2012). Although Birk et al. (2012) assessed only young male participants, their results suggest that lower-limb exercise can cause systemic adaptations to vascular function, which is likely caused by increases in shear stress. Therefore, it seems likely that the improvement in BA vascular function identified within the cross-sectional studies is due to systemic vascular adaptations from many years of lower-limb exercise training.

Additionally, it has been previously shown that vascular function in older females decreases at a faster rate than in males (Celermajer et al., 1994). As the current systematic review and meta-analysis included both male and female participants, it is possible that our results could have differed if males and females were analyzed separately. However, there were too few studies containing female participants to split the analysis by sex. Furthermore, it has been previously found that estrogen may be required to induce the benefits of endurance exercise on vascular function, potentially by increasing NO bioavailability further (Moreau et al., 2013). As the females included in the meta-analysis were post-menopausal and not receiving estrogen supplementation, perhaps intake of the hormone after menopause alongside aerobic exercise may have helped to improve vascular function further. However, as the majority of the cross-sectional participants within the current meta-analysis were male, the results from the cross-sectional analysis may better represent the male population. Future studies may wish, where possible, to report male and female results separately, and to further report the menstrual status of female participants.

CONCLUSION

In summary, the current systematic review and meta-analysis identifies that aerobic exercise training during advancing age can maintain healthy vascular function compared with otherwise healthy sedentary peers. These findings emphasize the importance of remaining active throughout the life-span. However, currently there is not enough evidence to suggest that aerobic exercise interventions ranging from 2 to 12 weeks can improve vascular function in previously sedentary older adults. Nonetheless, sedentary older individuals should still be encouraged to become active until more evidence becomes available.

AUTHOR CONTRIBUTIONS

The literature search and selection of studies was performed by authors AC and AB. Following an initial screen of titles and abstracts (AC), full scrutiny of potentially eligible studies were independently screened by AC and AB using the specific inclusion criteria. NS arbitrated any disagreements in study inclusion. Study quality assessment was performed by AC. All authors contributed to the development of the final manuscript.

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Bouncing Back! Counteracting Muscle Aging With Plyometric Muscle Loading

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The preservation of muscle power is crucial in aging for maintaining mobility and performing daily tasks. Resistance training involving high movement velocities represents a valid strategy to slow down the rate of sarcopenia, counteracting the loss of muscle mass and muscle power. Plyometric exercise may represent an effective training modality for increasing muscle power; however, its application in older populations has been sparingly investigated, as the high impact actions involved may reduce its feasibility for older individuals. By adopting a safer modality of plyometric training, we investigated if a 6-week plyometric training intervention could increase knee extensor muscle size, architecture, force and power in 14 young (YM, age = 25.4 ± 3.5 y; means ± SD) and nine older males (OM, age = 69.7 ± 3.4 y). Volunteers trained 3 times/week using a device similar to a leg press machine where the user was required to bounce against his body mass on a trampoline. Pre-to-post training changes in isometric maximum voluntary torque (MVT), leg extension power and vastus lateralis (VL) architecture were assessed. Muscle power increased in both groups (+27% OM -$P < 0.001$, 20% YM -$P < 0.001$), although the total external work performed during the training period was significantly lower for OM (i.e., ∼-47%). Both groups showed significant increases in muscle thickness (MT) (+5.8 OM -$P < 0.01$ vs. +3.8% YM -$P < 0.01$), fascicle length (Lf) (+8% OM -$P < 0.001$ vs. +6% YM -$P < 0.001$), and pennation angle (PA) (+7.5% OM -$P < 0.001$ vs. +4.1% YM -$P < 0.001$). The current study shows that trampoline-based plyometric training is an effective intervention producing a rapid increase in muscle mass and power in both young and older individuals. The training modality used in this study seems to particularly benefit the older population, targeting the morphological and functional effects of sarcopenia in human muscle.

Keywords: aging, sarcopenia, dynapenia, stretch-shortening cycle, muscle architecture, muscle power, muscle remodeling

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INTRODUCTION

It has been estimated that 5–13% of people older than 60 years show a significantly increased rate of muscle mass loss (Morley et al., 2014). This prevalence escalates to 50% in people over 80 years indicating that physiological muscle loss in aging drastically accelerates between the age of 70 and 80 years (Morley et al., 2014). This progressive age-related decrease in muscle mass, associated with a loss of muscle strength, represents the current definition of sarcopenia (Cruz-Jentoft et al., 2010; Mitchell et al., 2012) a geriatric syndrome predisposing to poor outcomes such as mobility disorders, disability, poor quality of life, and increased risk of death (Cruz-Jentoft et al., 2010). Critically, the loss of muscle strength is far greater than that of muscle size, and at the age of 80 years it is four-fold greater (Moore, 2014). In today's population, with a proportion of elderly citizens exceeding that of young people, this is a real concern. Considerably more so since impaired muscle function has been shown to be a predictor of hospitalization, disability, and mortality (Roubenoff and Hughes, 2000; Metter et al., 2002; Delmonico et al., 2007; Cruz-Jentoft et al., 2010; Guadalupe-Grau et al., 2015).

Resistance exercise has widely been advocated among the best strategies for counteracting the decrease of skeletal muscle mass in older age; however, there is no consensus on the optimum form of exercise. In fact, the general opinion is that the frequency, intensity, volume, and/or mode of the optimal exercise warrant further investigation into how best prevent muscular decline in aging individuals. What is agreed, however, is that preservation of muscle power (the ability to accomplish muscular work per unit time) is fundamental for combating the decrease of physical performance associated with sarcopenia (Fielding et al., 2011; Quinlan et al., 2017). In this regard, the preservation of strength and velocity of movement in older adults is closely related to the ability to generate muscle power, which is fundamental for maintaining mobility and for the performance of simple daily tasks such as negotiating a flight of stairs, raising from a bed or chair or walking to the grocery store (Miszko et al., 2003; Reid and Fielding, 2012). Unfortunately, with aging, muscle power declines at a faster rate than muscle strength (Skelton et al., 1994; Izquierdo et al., 1999; Reid and Fielding, 2012; Alcazar et al., 2017; Cadore and Izquierdo, 2018). In fact, Evans (Evans, 2000) stated that a decrease in muscle power has more significant implications for risk of hip fracture, performance in daily tasks, and functional independence than a decrease in strength. Therefore, it seems imperative that maintenance of power through rapid force-generating exercises should be implemented in exercise programs for the elderly (Cadore et al., 2018).

In the last decades, plyometric exercise has become increasingly popular for increasing muscle power (Markovic and Mikulic, 2010). Plyometric training utilizes the stretch-shortening cycle (SSC), a term that describes movement tasks where the muscle-tendon unit is stretched and then shortened rapidly (Cardinale et al., 2011), and in which the elastic properties of a muscle are involved to generate maximal power production. The use of plyometrics within the older population has previously been advocated as a potential preventive measure against sarcopenia (Faulkner et al., 2007). A major problem that hampered the application of such programs is that plyometric exercises involving repeated cycles of fast deceleration followed almost immediately by rapid acceleration of the body in the opposite direction seem hard to perform for older individuals without risk of injury. To circumvent this problem, in the present study we used the Tramp-Trainer machine (FREI AG, Hinterzarten, Germany, EU) consisting of a trampoline attached to an inclined sledge, enabling the performance of repeated plyometric jumps while the subject is sitting on a chair with the back fully supported. With this device, exercise is performed seated and with a defined trajectory, which may reduce injury risk.

Hence the aim of the present study was to test the hypothesis that plyometric training, based on exercise against one own's body mass, would be effective for increasing muscle mass and power in older individuals.

MATERIALS AND METHODS

Fourteen young participants (height = 176.1 ± 6.3 cm, mass = 72.2 ± 13.8 kg, age = 25.4 ± 3.5 years (20-32 years range); data expressed as means ± SD) and 9 older volunteers (height = 172.1 ± 3.1 cm, mass = 79.1 ± 6.8 kg, age = 69.7 ± 3.4 years (65-76 years old range)) were recruited to undergo a 6-week plyometric training program. All participants were healthy, fully independent and recreationally active but not practicing regular vigorous physical activity and had not been engaged in any plyometric or strength training programs within the past 6 months. All participants were medically screened by means of a medical questionnaire to exclude sufferers of joint disease and metabolic, respiratory or cardiovascular impairments. All subjects provided written, informed consent. This study was carried out in accordance with the Declaration of Helsinki. The protocol was approved by The University of Nottingham Ethics Committee.

Trampoline-Trainer Exercise

Training was performed on the "Tramp-Trainer" (TT) exercise machine (**Figure 1**) (FREI AG, Hinterzarten, Germany, EU). Briefly, the TT is a device similar to a leg press machine, except that the user is required to flex and extend the lower limbs (supported by two ankle braces connected to a spring, as shown in **Figures 1A,B**) against his/her own body weight on a trampoline, and thus on a compliant and elastic surface. The user is seated in a chair attached to a 1.5 m carriage with adjustable inclination, which allows the workload to be modified. In the current study, inclination was set to 22° (maximum incline) to elicit the greatest possible response to exercise. Essentially, the exercise on the Trampoline Trainer started from a semi-squat position with the knees flexed to a range between 90° and 80° (0° = anatomical zero/full leg extension) followed by a maximal push of the lower limb muscles (hip extensors, knee extensors and plantar flexors). The body and chair are then displaced along the rail during the jump, followed by landing on the elastic trampoline and immediate recoil as the subject bounces back; the entire cycle is then repeated for successive jumps (**Figure 1D**).

FIGURE 1 | (A,B) Trampoline Trainer exercise device. **(C)** The red arrow points to the meter scale applied to the rail track of the TT device. **(D)** Representation of a bounce sequence performed by an elderly volunteer.

Training Protocol

Training was performed 3 times per week for a period of 6 consecutive weeks. Training volume was based upon the guidelines of (Chu, 1998), stating that beginner and intermediate status athletes should not exceed 120 foot contacts per session when implementing a new plyometric training program. In the young group, training volume was therefore fixed at 4 sets x 30 repetitions for the first 4 weeks, followed by 5 sets × 30 repetitions for the final 2 weeks. In the elderly group, training volume was fixed at 3 sets × 30 repetitions for the first 4 weeks, followed by 4 sets × 30 repetitions for the final 2 weeks. From pilot data it became obvious that the initial increase in training volume after 2 weeks was too strenuous for the elderly group to manage, hence in both populations the training volume was increased after 4 weeks, rather than after 2 weeks.

Training load was matched across subjects by determining 30 Repetition Maximum value before the start of the program. This load equates to that which subjects could perform no more than 30 repetitions within a set without a decrease in bounce performance. A graduated scale marked in centimeters was fixed on the inside side of the rail-track (**Figure 1C**) of the Tramp Trainer to monitor bounce height during the exercise. Thus, 30-RM was first tested. Then, during the training protocol, subjects were instructed to bounce to a height that corresponded to their 30-RM, and if they fell 5 cm or more below their 30-RM height for up to 3 consecutive bounces, they were prompted to increase bounce height in order to maintain a constant training load.

As training progressed, loading increased progressively to maintain the training load at 30RM. The 30RM values were re-assessed every 7 days (before the start of the first session each new training week), thus new 30RM jump height values were identified. When volunteers reached the highest value of the meter scale attached to the carriage (i.e., the top of the carriage) for ∼90% of the repetitions in a single training session, in order to increase the training load, a 15 kg weighted vest was provided from the successive session in order to further increase the training load. Successively, training load increase was provided by increasing the number of series from week 4 of the training period.

During a familiarization session on the TT, each participant practiced starting and landing in repeated jumps from a knee angle of ca. 90° measured with manual goniometer (full knee extension was taken as anatomical 0). During each training session a red mark was placed on the side of the inclined plane rail-track as a visual target corresponding to a knee flexion of ca. 90°.

Isometric Maximum Voluntary Contraction Torque

Isometric muscle torque was measured using an isokinetic dynamometer (Cybex Norm, Cybex International Inc., NY, USA)

at a joint angle of 70°, with full extension corresponding to 0°. After a brief warm-up, subjects performed two maximum voluntary contractions, which lasted for 4 s, with a rest period of 30 s between contractions. Subjects were provided with real time visual feedback of torque production during isometric contractions. The maximum isometric torque value (MVCT peak) was chosen for data analysis.

Muscle Power Test

Leg extension power was assessed using the Nottingham Power Rig (Nottingham University, Nottingham, UK). The power rig provides a measure of peak power (W) generated by the lower limbs during a hip and knee extension movement (Bassey et al., 1992). The specifics of such device are described in detail by Bassey and Short (1990). Briefly, the device consist of a seat, and a lever (on which the feet are placed in order to exert force) which is connected to a flywheel by a chain. The leg extension movement is completed in 0.25–0.40 s (Bassey and Short, 1990). The resistance to the movement is minimal and remains nearly constant throughout the whole movement (Bassey and Short, 1990). During the testing sessions of the present study, subjects were asked to push as hard and as fast as possible (i.e., at their maximum velocity) on the raised foot plates through the full range of movement (5 repetitions). If subjects showed progressive improvements in peak power in the 5 repetitions, unlimited efforts were allowed until a plateau of performance was reached.

Total External Mechanical Work

The average total external mechanical work ($W_{ext,}$ kj) performed throughout the study by both young and elderly group was calculated. Individual W_{ext} was calculated from body mass (plus 15 kg if subject were wearing the weighted vest), TT inclination, and average height reached during each bounce performed in each session (relative to the maximum height of the track/carriage).

For a single set of 30 bounces, W_{ext} was calculated as follows:

$$W_{ext}(kj) = [(BM + VM)xgx(\sin 22)]x(1.265 - h_{ex})x30 \quad (1)$$

where BM (kg) represents the subject body mass, VM (kg) the mass of the weighted vest, g is the gravitational acceleration (9.81 m^*s^{-2}) multiplied by the sine of the pre-set inclination of the track, 22°, which was kept constant throughout the whole study), h_{ex} (m) represents the average height reached by every bounce in the set (relative to the total length of the track, 1.265 m), and 30 is the number of bounces performed per set.

Muscle Morphology and Architecture

Muscle architecture of the vastus lateralis muscle was measured *in vivo* using B-mode ultrasonography (Mylab70, Esaote, Genoa, Italy) (**Figure 2**). The measurements were acquired not more than 7 days before the start of the training period (in which the participants were asked to refrain from any occasional strength training session or strenuous exercise) and between 3 and 5 days after the last training session. Ultrasound images were obtained from the subjects' right leg when they were resting in a supine position. Vastus lateralis fascicle length (Lf), pennation angle (PA), and thickness (MT) were measured using a 10 cm, 10–15 MHz, linear-array probe, according to the method described in detail by Franchi et al. (2018b). Briefly, the probe was positioned over the belly of the vastus lateralis, carefully adjusted to the fascicle plane while the minimal pressure was applied (as described Franchi et al., 2017b). Ultrasound scan images were then analyzed using ImageJ image analysis software. Lf was determined through extrapolation of fibers and aponeuroses if a portion of the fascicle extended outside of the captured ultrasound image (as described Franchi et al., 2014, 2018a). PA was measured at the intersection between the fascicles and the deep aponeurosis. MT was measured as the perpendicular distance between the superficial and deep aponeuroses (Franchi et al., 2017b).

EMG Evaluation During a Single Counter-Movement Jump (CMJ) Task

Electromyographic activity (EMG) was recorded from the vastus lateralis during a maximal vertical jump testing (CMJ). This design was adopted as CMJ is very similar to the movement performed on the training device and also because it represents a valid and well standardized performance test. Bipolar (20 mm inter-electrode distance) surface EMG recording was employed using two electrodes placed 94 ± 13.2 mm along a line from the superior lateral side of the patella, to the anterior superior iliac spine, starting from the patella (Rainoldi et al., 2004). The ground electrode was placed on the lateral side of the patella. Before positioning the electrodes, the skin was shaved, gently abrased using emery paper, and cleansed using alcohol swabs to reduce impedance below 5 KΩ. Raw EMG signal was acquired with a sampling frequency of 1000 Hz and processed with a multichannel analog-digital converter (EMG 100C, Biopac systems inc., Santa Barbara, CA, USA). The raw signal was then filtered using high- and low-pass filters set at 10 and 500 Hz, respectively, digitized, stored and analyzed by Acqknowledge software (Biopac systems inc., Santa Barbara, CA, USA). Root mean square (RMS) during a CMJ was calculated over a 200-ms time frame which corresponded to ±100 ms from the RMS peak, taking into account the electromechanical delay (EMD). This was set at 43.4 ms for the elderly group (Reeves et al., 2004), and 32.8 ms for the young group (de Boer et al., 2007). EMD is defined as the delay between the onset of electrical activity and torque. The onset of electrical activity was defined as an increase of 15 mV above baseline (Reeves et al., 2004).

Rate of Perceived Exertion (RPE) and Muscle Soreness

Rate of perceived exertion (RPE) was monitored using the Borg Scale (Borg, 1998) at the end of each training sessions; participants were asked to visually indicate the level of general fatigue perceived. In addition, muscle soreness of the lower limbs was monitored using the soreness scale (Cook et al., 1997), before the start of every training and after each set (4 times/training) (immediate soreness after performing a set of 30 maximal bounces).

Visual supports representing each level of the scale (from 1 to 10 for both scales) were provided to help volunteers familiarizing

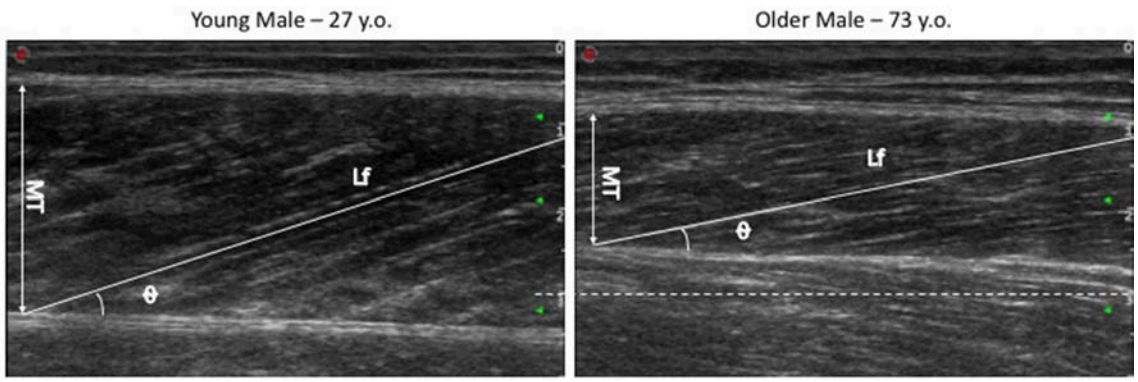

FIGURE 2 | Vastus lateralis US images of Young vs. Old volunteers (acquired with same scale and US preset parameters). The dotted white line represents the 3 cm scale value, highlighting that images share the same starting point (0 cm) and that the representative image from the old volunteer shows a significantly smaller MT compared to the young participant.

with the scale itself and expressing the correct value of fatigue or soreness when asked.

Blood Pressure and Heart Rate

Because of the novelty of such training modality, for safety reasons, only the OM group was monitored during training by a medical doctor who checked, before and after each set, the heart rate (HR) and diastolic and systolic blood pressure (BP). Parameters were recorded in a "health monitor diary" and average values were calculated for week 1 and week 6 of the training period.

Statistical Analysis

Differences in the functional, morphological, and architectural muscle components of the study between groups (young vs elderly) and time (baseline vs post training) were investigated using a two-way repeated measures ANOVA. Bonferroni's multiple comparisons test was used to identify significance within groups from baseline to 6 weeks (post training). Differences between groups in the total external mechanical work and pre-to-post training increase percentage in MT and Power (i.e., also normalized by work) were evaluated by an unpaired Student's *T*-test. GraphPad Prism software (version 7.0; GraphPad software Inc. San Diego, CA) was used to perform all statistical and *post hoc* analysis.

RESULTS

The average values for functional and morphological adaptations of each group at each time point are presented in **Table 1** (means ± S.D).

Isometric Maximum Voluntary Contraction Torque

The average group values for Isometric MVCT were greater after 6 week of plyometric training compared to baseline in both young (246.2 ± 50.7 Nm vs. 260.4 ± 50.2 Nm, $p = 0.09$) and older males (204.8 ± 44 Nm vs. 221.9 ± 40.4 Nm, $p = 0.13$) (increase percentage values = 6.8% ± 12.6 -YM vs. 8.6% ± 9.9% -OM). Due to the high individual variance of such responses, such increases in MVCT were not significant, neither between time points nor between groups.

Maximum Leg Extension Power

Muscle Power (leg extension power) increased significantly after the 6-weeks training period compared to baseline in both young and older male groups (423.9 ± 136.4 W vs. 506.1 ± 153.2 W, $P < 0.001$, and 327.3 ± 82.1 W vs. 408.2 ± 108 W, $P < 0.001$, respectively) (increase percentage values = 20% ± 11, $P < 0.001$ -YM vs. 27% ± 11, $P < 0.001$ -OM), without significant differences observed between groups.

Muscle Architecture

Vastus lateralis fascicle length (Lf) presented a significant increase in both groups after training (YM = 7.66 ± 0.9 cm vs. 8.12 ± 0.9 cm, $P < 0.001$, and OM = 7.51 ± 0.4 cm vs. 8.15 ± 0.4 cm, $P < 0.001$) (increase percentage values = 6.1% ± 3.1%, $P < 0.001$ -YM vs. 8% ± 2.5%, $P < 0.001$ -OM) without significant differences observed between groups. Baseline values were not significantly different between groups.

Pennation angle (PA) presented significant changes in both groups (YM = 16.4 ± 1.4° vs. 17 ± 1.6°, $P < 0.01$, and OM = 14.5 ± 2.3° vs. 15.5 ± 2.2°, $P < 0.001$) (increase percentage values = 4.1% ± 3.4%, $P < 0.01$ -YM vs. 7.5% ± 3.2, $P < 0.001$ -OM). The increase was significantly greater in OM compared to YM ($P < 0.05$). Baseline values were significantly different between groups ($P<0.05$); however, this between-groups difference was lost at the 6-week time point.

Muscle thickness (MT) increased significantly pre-to-post training in both groups (YM = 2.44 ± 0.3 cm vs. 2.54 ± 0.3 cm, $P < 0.01$, and OM = 1.96 ± 0.3 cm vs. 2.09 ± 0.3 cm, $P < 0.01$) (increase percentage values = 3.8% ± 3.4%, $P < 0.01$ -YM vs. 5.8% ± 5%, $P < 0.01$ -OM) without significant differences observed between groups. Baseline absolute values were significantly different between groups ($P < 0.05$) and so they remained at the 6-week time point ($P < 0.05$).

TABLE 1 | Pre-to-post values for muscle functional and morphological features.

	Young males		Older males	
	Baseline	**6 Weeks**	**Baseline**	**6 Weeks**
MVC (Nm)	246.15 ± 50.69	260.35 ± 50.16	204.75 ± 43.98	221.03 ± 42.87
Power (W)	423.83 ± 136.33	506.08 ± 153.23***	327.26 ± 82.08	408.21 ± 107.97***
CMJ EMG (mV)	0.23 ± 0.10	0.30 ± 0.13**	0.15 ± 0.06	0.21 ± 0.07*
VL MT (cm)	2.44 ± 0.34	2.54 ± 0.35**	1.96 ± 0.32^	2.09 ± 0.33**,^
VL PA (deg)	16.35 ± 1.36	17.03 ± 1.63***	14.48 ± 2.32^	15.51 ± 2.08***
VL Lf (cm)	7.66 ± 0.90	8.12 ± 0.91***	7.51 ± 0.41	8.15 ± 0.39***
RPE	5.18 ± 1.53	3.33 ± 1.81**	4.45 ± 1.91	3.65 ± 1.62
Soreness scale	3.33 ± 1.84	1.49 ± 1.82**	1.85 ± 2.01	1.21 ± 1.73

*Baseline vs. 6 Weeks differences: *P < 0.05; **P < 0.01; ***P < 0.001.*
Between groups differences (at the same time-point): ^P < 0.05, ^^P < 0.01.

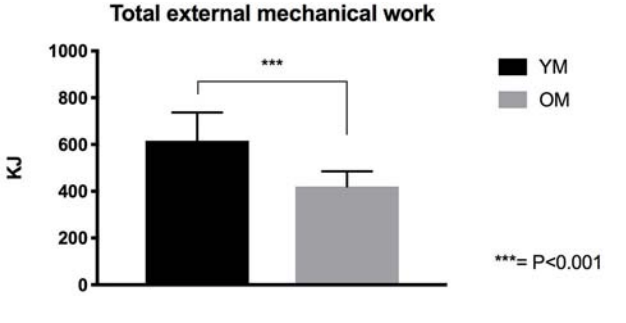

FIGURE 3 | Total External mechanical work performed by YM and OM groups.

EMG RMS During a CMJ task

EMG RMS evaluated during a maximal CMJ presented a significant in both groups after the training program. The changes were similar in both young and older groups, as YM showed a 42% increase ($P < 0.01$) and the OM showed an increase of 46% ($P < 0.05$) compared to baseline values.

Total External Mechanical Work Performed

Total external mechanical work performed in the 6-week training period was found significantly different ($P < 0.001$) between YM and OM groups. The average total work performed by the YM was 616.1 ± 121 kJ while that performed by the OM was 420 ± 65.2 kJ. Thus, over the 6-week training period, YM performed 46.6% more work than the OM group (**Figure 3**).

Functional and Morphological Adaptations Expressed by Total External Mechanical Work Performed

When muscle power and MT percentage increase values were normalized by the total external mechanical work performed, a statistically significant difference was observed between YM and OM (power = 10.4% ± 4.6% vs. 18.5% ± 9.1%, respectively – $P < 0.05$; MT = 1.7% ± 1.8% vs. 4.4% ± 3.7%, respectively – $P < 0.05$). MVC did not clearly changealso when normalized for total external mechanical load (**Figure 4**).

RPE and Soreness Scale

RPE and soreness scale values did not differ between YM and OM groups. However, both RPE and soreness scale values showed a significant decrement in the YM group from week 1 to week 6 time points ($P < 0.01$) (**Table 1**).

Monitored Cardiovascular Parameters (Older Male Group Only)

In the 9 Older Males, heart rate (HR) and diastolic and systolic blood pressure (BP) were monitored before and after the training session on week 1 and week 6 of the training period. Pre and post-exercise session HR did not significantly change from week1 to week6 time points (pre-exercise session = 67.8 ± 33.2 vs. 67.7 ± 38 bpm, respectively; post-exercise session= 128.7 ± 17.4 vs. 126.1 ± 16 bpm, respectively). No significant differences were observed for pre-exercise session systolic and diastolic BP between week 1 and week 6 time points (140.4 ± 3.6/84.3 ± 10 mmHg vs. 136.5 ± 11.9/83.1 ± 9.6 mmHg, respectively). A strong trend ($P = 0.06$) was observed for significant reduction of post-exercise session systolic BP from week1 to week 6, whereas diastolic BP did not show any statistically significant differences with time (199.5 ± 18.7/95.3 ± 14.1 mmHg vs. 179.1 ± 24.4/89.4 ± 11.4 mmHg, respectively).

DISCUSSION

In this proof-of-concept study, we investigated whether plyometric exercise could be used as an efficient strategy to counteract sarcopenia in the elderly, as this may be expected to positively impact on mobility and quality of life (Evans, 2000). Accordingly, we investigated skeletal muscle morphological and functional adaptations to a plyometric training intervention in older and young individuals. The main findings show that plyometric exercise produces significant and rapid improvements in muscle power and size in both young and older participants. Knee extensor muscle power (measured through a maximal velocity knee extension on the Nottingham Power Rig device) increased by 27% in OM and 20% in YM after just 6 weeks of training. This observation seems extremely noteworthy as it shows that a substantial improvement of muscle power may be

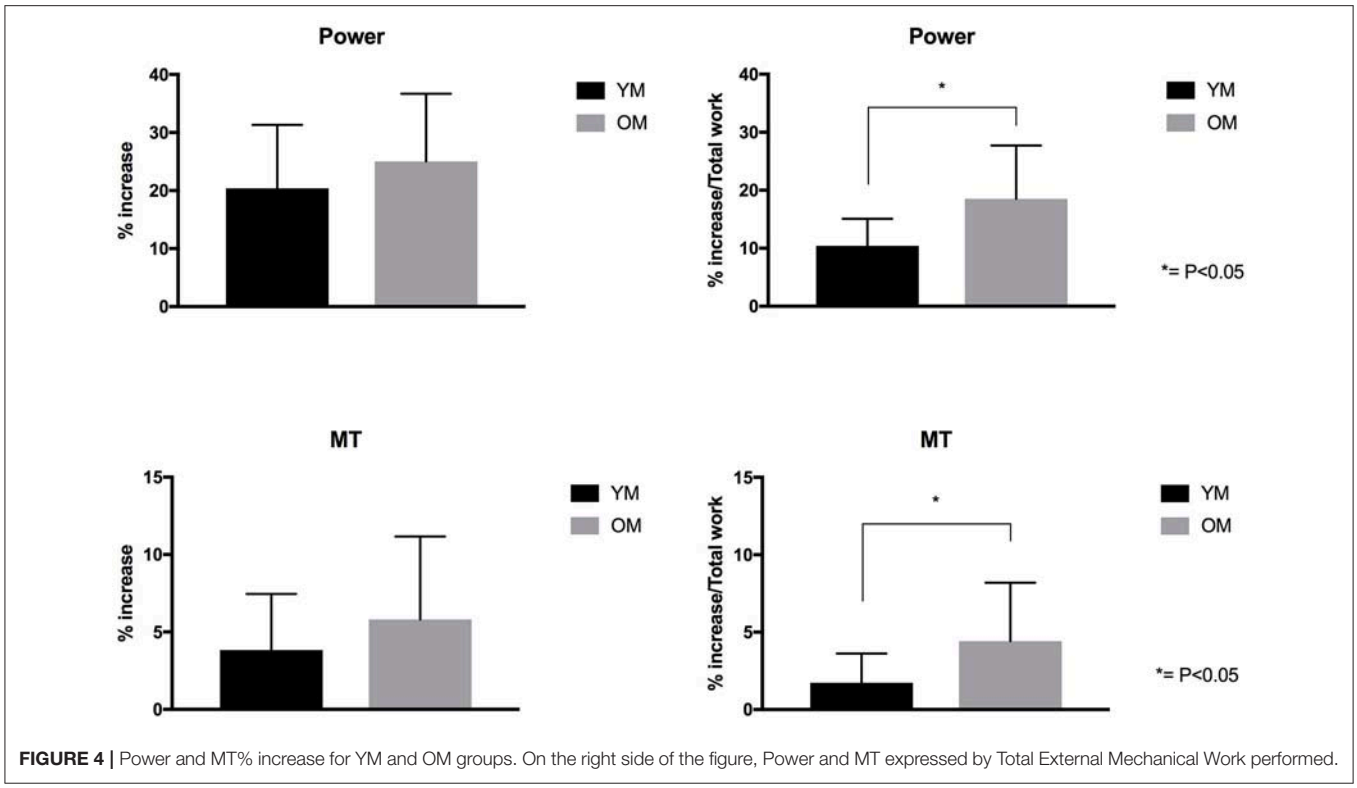

FIGURE 4 | Power and MT% increase for YM and OM groups. On the right side of the figure, Power and MT expressed by Total External Mechanical Work performed.

achieved in less than 2 months of resistive training. Interestingly, expressing this gain as daily rate, this equates to 0.64%/day for the OM group. This is identical to the percent daily gain which may be estimated from the study of Izquierdo et al. (2001), reporting a 36% increase in leg extensors muscle power after 8 weeks of heavy resistance training in 64-year old men, equating to a daily gain of 0.64%/day. Hence plyometric training seems to produce very similar gains in muscle power as those achieved by heavy resistance training over a period of 6–8 weeks of training.

Since mechanical power is the product of force and velocity, an increase in power output may be the result of an increase of muscle force, velocity or both. As the results of this study show, maximum isometric torque did not significantly change after the training in either OM nor YM, however fascicle length did in both OM (+8%) and YM (+6%) participants. Theoretically, assuming that the increase in fascicle length is reflective of an increase in fiber length due to an increase in sarcomere number (Williams, 1990), and assuming an optimum sarcomere length for the VL muscle of 2.73 μm (0.000273 cm) (Walker and Schrodt, 1974), then the observed fascicle length of 8.15 cm after training and 7.51 cm before training for OM and 8.12 cm after training and 7.66 cm before training for YM result in an estimated increase in sarcomere number of 2344 (8.15/0.000273 cm minus 7.51/0.000273 cm) for the OM and of 1685 (8.12/0.000273 minus 7.66 /0.000273) for the YM. We advise the reader to interpret this simplified mathematical calculation with caution, as recent work in animal (Moo et al., 2016, 2017) and human muscle (Lichtwark et al., 2018) has showed that sarcomere lengths are non-uniform within distinct muscle regions (Moo et al., 2016; Lichtwark et al., 2018) and even more inhomogeneous upon contraction (Moo et al., 2017). Therefore, this could represent a limitation of our mathematical simplification. Nonetheless, since the muscle maximum shortening velocity has been shown to be proportional to the number of sarcomere in series (Bodine et al., 1982), the estimated increase in sarcomere number may have contributed to the observed changes in muscle power. However, this contribution (8% in OM and 6% in YM) clearly does not fully account for the increase in muscle power (27% in OM and 20% in YM). Nonetheless, as our findings show, the increase in muscle power was accompanied by a significant increase in EMG activity (RMS) of the VL muscle (42% in YM and 46% in OM during CMJ). This finding suggests that improved muscle recruitment may have contributed to the increase in muscle power induced by the plyometric intervention of this study.

While a small but significant increase in MT in both OM (5.8%) and YM (3.8%) was observed, the increase in muscle strength (8.6% in OM and 6.8% in YM) did not reach statistical significance despite the presence of muscle hypertrophy. The increase in MT predicted a proportionate increase in muscle strength. It seems likely that the lack of torque increase reflected the type of resistive training used in this study, which was focused on muscle power rather than muscle strength, as gains in muscle strength have been shown to be training-specific (Tillin et al., 2012). In addition, the lack of significant changes observed for MVCT could reflect the non-matching movement specificity between the isometric MVCT and the TT training device.

A key outcome of the study is that, while both groups presented similar increases in MT and muscle power, OM

performed significantly less total amount of external mechanical work compared to YM (∼-47%) over the 6-week training period. Thus, by normalizing MT and power percentage increase values by the total work performed, such changes were markedly greater in the older population (**Figure 4**). The observation that OM achieved very similar hypertrophic responses to those in YM but while performing fewer repetitions/sets per week, with no use of the weighted jacket (i.e., no supplemental overload than the body weight), and consequently while performing less total external mechanical work compared to YM, is indeed noteworthy. This observation could indeed suggest that this training modality may result optimal for an elderly population. In addition, the increase in MT, expressed for the external work performed, presents a significant discrepancy of ∼2.7% between age groups in favor to the elderly participants. However, when expressing the increase in muscle power for the total external work performed, the OM group showed a significantly greater capacity for augmenting in power (∼8.1% discrepancy between groups) in the same time-frame. In addition, these responses were achieved after just 6 weeks of plyometric-based training, which represents a rather efficient time frame in rehabilitation and clinical settings. Previous research has shown that decreased muscle mass is associated with an increased risk of hip fracture (Cummings et al., 1995; Turner et al., 2004), and that, concomitantly, a marked reduction in power production represents a major risk factor for hip fracture among the elderly (Phillips et al., 1998). This has particularly important implications for sarcopenic populations, in which a decline of power has been observed faster than declines in isometric strength (Izquierdo et al., 1999) and knee extensor strength (Skelton et al., 1994). Leg muscle power has indeed been shown to be more important for performing daily activities than strength in frail elderly people (Bassey et al., 1992). The increase in power seen following plyometric exercise is thus likely to have positive implications for improving daily activities such as stair climbing, time to rise from a chair/toilet, and walking around the home. Bassey et al. (1992) showed that older men and women who performed these tasks with assistive aids had 42–54% less leg extensor power compared to those who performed the tasks without aids. Thus, the present findings suggest that this novel plyometric protocol may represent a time-efficient strategy to counteract sarcopenia in the aging population.

When considering skeletal muscle performance and aging, changes in the structure of muscular system undoubtedly acquire an important role, as architectural remodeling of skeletal muscle can influence its functional properties (Narici and Maganaris, 2007). In the present study, both YM and OM groups presented significant changes in muscle architecture (i.e., MT, PA and Lf). In the elderly group, the aforementioned increase in MT (5.8%) and in fascicle length (8%) was accompanied by increases in pennation angle (7.5%). These architectural adaptations are evidence of a hypertrophic response (i.e., increase in MT) and could reflect distinct strategies of new contractile material deposition (Franchi et al., 2017a), since an increase in PA may reflect the addition of sarcomeres in parallel (Narici et al., 2016) and an increase in Lf is indicative of a potential addition of sarcomere in series (Franchi et al., 2014, 2016). The YM group also presented significant changes in MT (3.8%), Lf (6.1%), and PA (4.1%, significantly less than the increase in OM); however, compared to the OM group, slightly weaker architectural responses to this specific training modality were observed in YM. These results warrant some considerations.

The significant different increase in PA between age groups suggests that the intensity of the training stimulus may not have been sufficiently high in the YM to elicit a greater response, especially considering that the young participants were not presenting any signs of sarcopenia to begin with, and therefore not as sensitive as the elderly to the training intervention (Komi, 2003). The increase in fascicle length following plyometric training seems to be in line with the work of Blazevich et al. (2003). The observation that the both the YM and OM group showed a similar strong increase in Lf may suggest that the eccentric phase of the stretch-shortening cycle have been the main driver of hypertrophic responses (i.e., the adaptations reflect the predominant fascicle behavior seen during the training). Theoretically, during the SSC the muscle is firstly stretched (deceleration phase–eccentric component) before it rapidly contracts concentrically (acceleration phase) (Bosco et al., 1981). However, it is still debated if the muscle undergoes a full eccentric phase during plyometric actions, as some work suggested that the tendon and the series elastic components are mostly lengthened, while the muscle fascicles behave "quasi-isometrically" (Finni et al., 2003; Ishikawa and Komi, 2004). Although there is evidence of significant (Ishikawa et al., 2006) (or a tendency for – Hirayama et al., 2017) fascicle lengthening when using high load, this mechanical stretch applied onto the fascicles may not be comparable to that observed in pure eccentric contractions (Franchi et al., 2014). Nevertheless, it has been previously shown that isometric contractions at longer muscle lengths (∼90°) could lead to an increase in Lf (Noorkõiv et al., 2014). Lastly, the aponeuroses may have contributed to such adaptations in Lf, as they would have undergone longitudinal and transversal stretch during the SSC task, consequently resulting in significant stress applied onto the inserting fascicle (Raiteri, 2018). These events would in turn potentially trigger mechanotransductor signaling pathways, which have been recently associated to changes in muscle architecture (Franchi et al., 2018a). Taken together, all the aforementioned factors may play a determinant role in the remodeling of skeletal muscle following plyometric training in young and athletic populations.

Notably, these morphological changes were achieved in a relative short training period. Early muscle architectural remodeling has been previously documented in response to conventional, isokinetic and isoinertial resistance training (3–6 weeks duration) in young and older population (Blazevich et al., 2003, 2007; Seynnes et al., 2007; Franchi et al., 2015, 2018a; Brook et al., 2016) but they were not yet reported in response to plyometric training modalities, especially in older and/or sarcopenic men.

It is worth noting that the use if plyometric training for improving muscle mass, strength, power and endurance in old age has been previously advocated by Professor John Faulkner and colleagues (Faulkner et al., 2007), and our study seems to confirm the suitability of this type of exercise form improving

muscle mass and power in old age. Up to now, only one study investigated the neuromuscular adaptations to a 12-weeks plyometric program in older adults (Piirainen et al., 2014). Piirainen and colleagues compared a plyometric training modality to a more classical pneumatic resistance training in older adults (60–70 years old). Plyometric training was performed on a custom-built sledge ergometer very similar to the TT device used in this study, with the only difference that the platform surface was hard and not compliant (Piirainen et al., 2014). Whereas knee extensor MVC significantly increased after just 4 weeks of pneumatic training, significant strength increase was only observed after 12 weeks in the plyometric group: this is in agreement with the lack of significant changes in MVC of the present study, that is MVC changes probably reach significance after 6 weeks of training.

A note of caution, however, ought to be expressed on the use of plyometric training. As it involves of repeated stretch -shortening contractions, the training program should be designed by certified exercise professionals in order to minimize the risk of "contraction-induced injury" to participants. Although in this study we did not measure muscle damage markers such as creatin kinase, significant muscle damage may be excluded as neither our older or younger participants reported an increase in the muscle soreness scale or any adverse effect after each exercise session.

CONCLUSION

The current study showed that plyometric exercise is an effective tool in counteracting the morphological and functional effects of sarcopenia in human muscle in a training period of only 6 weeks. Although the total amount of external mechanical work was ∼47% lower in the elderly group, both young and older individual achieved similar increases in muscle size and power through plyometric exercise. Thus, we have successfully demonstrated that in a group of elderly subjects, plyometric exercise can increase muscle power in a time-efficient manner. This may have significant implications for reducing risks of hip fractures, improving ability to perform daily tasks, and improving functional independence and to maintain quality of life in elderly individuals.

DATA AVAILABILITY

The datasets generated for this study are available on request to the corresponding author.

AUTHOR CONTRIBUTIONS

MF, NR, and MN conceived the study. MF, MN, NR, and AC contributed to the study design assembly. MF, EM, AC, JQ, and PH perform the study and the data collection. MF, EM, and AC performed the data analysis. MF, AC, EM, and MN contributed to the data interpretation. MF, AC, EM, and MN drafted the manuscript; all authors approved the final version of the manuscript.

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frontiers in Physiology

ORIGINAL RESEARCH
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Delayed Reperfusion—Coronary Artery Reperfusion Close to Complete Myocardial Necrosis Benefits Remote Myocardium and Is Enhanced by Exercise

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The present study aimed to analyze the effects of reperfusion of a distant coronary artery on cardiac function, the ultrastructure, and the molecular environment of the remote myocardium immediately after the completion of myocardial regional necrosis: delayed reperfusion (DR). Additionally, the effects of prior exercise on the outcomes of DR were investigated. Female rats with permanent occlusion or delayed reperfusion were randomly assigned to an exercise (swimming, 1 h/day, 5 days/week for 8 weeks) or sedentary protocol. Thus, the study included the following four groups: sedentary permanent occlusion, exercise permanent occlusion, sedentary delayed reperfusion, and exercise delayed reperfusion. The descending coronary artery was occluded for 1 h. Reperfusion was confirmed by contrast echocardiography, and the rats were observed for 4 weeks. Permanent occlusion and DR caused similar myocardial infarction sizes among the four groups. Interestingly, exercise significantly decreased the mortality rate. Delayed reperfusion resulted in significant benefits, including enhanced hemodynamics and papillary muscle contraction, as well as reduced apoptosis and collagen content. Protein calcium kinetics did not change. Meanwhile, developed tension and the Frank–Starling mechanism were enhanced, suggesting that calcium sensitivity was intensified in myofilaments. Remarkable remote myocardial benefits occurred after distant DR, and prior exercise intensified cardiac recovery. Our findings provide valuable information about DR. Our data might explain the better clinical outcomes in recent studies showing that late reperfusion could improve heart failure in patients with myocardial infarction. In conclusion, DR has remote myocardial benefits, including inotropism enhancement, pulmonary congestion reduction, and collagen and apoptosis attenuation, which are enhanced by prior exercise.

Keywords: myocardial infarction, delayed reperfusion, late reperfusion, exercise, ventricular performance, inotropism, molecular biology

INTRODUCTION

Myocardial infarction (MI) is a common cause of morbidity and mortality (Li et al., 2013), and many studies have evaluated the pathophysiological events occurring after MI. Coronary intervention during the acute MI phase is considered important for mortality prevention, myocardial remodeling, and reduction in the incidence and severity of congestive heart failure (CHF). Early reperfusion has been shown to reduce areas of necrosis (Sadanandan and Hochman, 2000; Vetterlein et al., 2003; Armstrong et al., 2013; Jones et al., 2015). Earlier implementation of coronary reperfusion is associated with greater benefits for the heart (Hochman et al., 2006; Wang et al., 2014). It is advisable to perform coronary reperfusion within 6 h following coronary occlusion for the rescue of ischemic myocardial injury (Sadanandan and Hochman, 2000). Notwithstanding, there is no consensus about coronary reperfusion performed after 6 h. The recent European guidelines (Ibanez et al., 2018) consider reperfusion therapy as class 1A in all patients with a duration of <12 h and persistent ST-segment elevation with symptoms of ischemia. Nepper-Christensen et al. (2018) reported that substantial myocardial salvage could be achieved beyond 12 h of symptoms in patients with MI and signs of ongoing ischemia. Abbate et al. (2008), in a necropsy study, described lower ischemia and apoptosis perinecrosis in infarcted patients with an open coronary artery than in those with an occluded artery. Gierlotka et al. (2011) analyzed patients who underwent coronary angioplasty 12–24 h following the start of symptoms and described an improvement in mortality when reperfusion was performed 12 h after MI, in contrast to a conservative approach.

Medical practice after early-onset coronary reperfusion is surpassed is not yet well-established. There is a need to determine the most appropriate cardiac pathophysiologic sequence following coronary occlusion and subsequent reperfusion at different times after coronary occlusion. These conditions emphasize the value of transactional research to better understand cardiac evolution in this scenario.

Most of experimental research in myocardial reperfusion focus injury reperfusion as the main aim. It is numerous the number of papers determined to study early reperfusion. To our knowledge, only one experimental report assessed the cardiac effects of late reperfusion. Nakagawa et al. (2008) performed myocardial reperfusion in rats 24 h after coronary occlusion and noted morphological and functional benefits: myocardial scar was thicker, wall tension reduced, and dP/dt increased. Additionally, it appears that no study has focused on the remote myocardial effects of regional coronary reperfusion immediately after complete myocardial necrosis. Moreover, no previous study has assessed the effects of prior exercise on remnant myocardial tissue. The purpose of the present study was to analyze the effects of reperfusion on cardiac function, ultrastructure and molecular environment of the remote myocardium immediately after the completion of myocardial regional necrosis in rats. Since this set is so different from usual study of late reperfusion, we called this set as delayed reperfusion (DR). As no previous studies have assessed the effects of prior exercise on remnant myocardial tissue in delayed reperfusion, we also sought to evaluate the influence of prior exercise in the outcomes of DR.

To define the duration of ischemia in our protocol, we considered the results of the studies by Hedstrom et al. (2009) and dos Santos et al. (2009) showing that in rats, total necrosis due to coronary occlusion occurs in <1 h. In the rats, we conducted coronary reperfusion immediately after the assumed risk area accomplished total necrosis, that is to say, 1 h after coronary occlusion.

METHODS

Animals

Female rats (Rattus norvegicus var. albinus, 12 weeks old, weighing 180–220 g) were randomly assigned to swimming (1 h/day, 5 days/week, 8 weeks) or sedentary protocols and subjected to permanent occlusion or DR, generating four experimental groups: sedentary permanent occlusion (SPO), exercised permanent occlusion (EPO), sedentary delayed reperfusion (SDR), and exercised delayet reperfusion (EDR). This study was conducted in accordance with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (publication No. 85-23, revised 1985), the policies and regulations of the United Kingdom described in the Journal of Physiology (Grundy, 2015), and the Ethical Principles of the Brazilian College of Animal Experimentation (COBEA). The protocol was approved by the Research Ethics Committee of the Federal University of São Paulo (UNIFESP), Brazil (CEP 0341/08).

Exercise Training Protocol

Exercise training was performed in a swimming pool (diameter, 132 cm; depth, 80 cm) filled with tap water warmed to 32–34°C by a feedback-controlled electric heating coil. The water was maintained in continuous turbulence to provide uninterrupted exercise. To allow adaptation, swimming was limited to 10 min on the first day and increased by 10 min each day. Rats were subjected to 60 min/day of swimming, 5 days/week for 8 weeks, as described by Bocalini et al. (2010). In each exercise session, 8–10 rats were placed together in the swimming pool. During the exercise period, an age- matched sedentary control group was exposed to similar room noise and handling but remained in their cages during rest.

Surgery for Induction of MI and Ischemia/Reperfusion

After the first 8 weeks of exercise or physical inactivity, the animals were anesthetized with ketamine (50 mg/kg) and xylazine (10 mg/kg). Thoracotomy was performed, and the left anterior descending coronary artery was isolated and occluded ∼3 mm from the origin of the aorta, using a previously described method (Johns and Olson, 1954) for induction of MI. The ischemia/reperfusion protocol described by Himori and Matsuura (1989) was as follows. A prolene suture 6-0 was passed to isolate the coronary artery and its point prepared. Before occlusion, two cotton threads were introduced into the prolene thread loop. The ends of each cotton thread were

transfixed to each side of the chest wall to provide traction. Thereafter, the coronary artery was ligated, and the chest was closed. After 1 h of ischemia, with the chest still closed, the outer ends of the cotton threads were pulled to promote the opening of the coronary artery, releasing the flow of blood and promoting reperfusion. Reperfusion procedures were confirmed by echocardiography (ECHO). Five minutes before reperfusion, ECHO was performed to characterize the hypokinesia or akinesia of the left ventricle (LV) antero- lateral wall-dependent coronary occlusion. Following removal of the cotton traction threads, rats underwent ECHO with contrast (Definity, 0.2 mL, Bristol-Myers Squibb, New York, NY, USA) injected intravenously to confirm that coronary flow was restored. Animals with no reperfusion (n = 47/156, 30%) were excluded from the study. During 24 h following coronary occlusion 48/109 (44%) rats have died. Animals with MI smaller than 37% of ventricular endocardial perimeter (20/61; 37%) were only considered for analyses of mortality and were not used for functional, histomorphometric and molecular evaluations. Following these procedures, animals remained sedentary for 4 weeks until the final analysis, utilizing 12 rats in SPO; 12 rats in EPO: 8 rats in SDR and 9 animals in EDR.

Echocardiographic Measurements

ECHO analysis was applied 4 weeks after the MI procedure. Rats were anesthetized, and measurements were taken using a 12-MHz transducer connected to an HP Sonos-5500 echocardiograph (Hewlett-Packard, Philips Medical System, Los Angeles, CA, USA). MI size was evaluated from transversal two-dimensional views of the LV on the basal, mid-transversal, and apical planes. In the diastolic phase, measurements of the LV endocardial perimeter (EP) and the infarcted segment length (IS) were determined for each view. MI size for each segment (MIS) was expressed as a percentage of the LV perimeter and calculated as follows:

$$\mathrm{MIS}(\%) = \mathrm{IS}/\mathrm{EP} \times 100.$$

As in our previous studies (Helber et al., 2009; Santos et al., 2009), only rats with large infarctions (>37% of LV) were evaluated. The diastolic (DA) and systolic (SA) transverse areas of the LV were measured using two-dimensional images of the three parasternal transverse planes. The final value was the arithmetic mean of the measurements of the three views. Systolic function was analyzed using the shortening fraction (SF) of the three transverse planes and calculated as follows:

$$\mathrm{SF} = \mathrm{DA} - \mathrm{SA}/\mathrm{DA} \times 100$$

Pulsed Doppler analysis of the LV side of the mitral valve provided flow velocity data, which were utilized to determine diastolic function parameters (E waves, A waves, and the E/A ratio).

Hemodynamic Analysis

The LV pressure of anesthetized rats was obtained by catheterization of the right carotid artery using a Millar catheter (Microtip®, 2F, Millar Instruments, Inc. Houston, TX, USA). Data were obtained using AcqKnowledge® 3.7.5 software (Biopac System Inc, Los Angeles, CA, USA) to compute the instantaneous LV systolic (LVSP) and diastolic (LVDP) pressures, heart rate, and positive (+dP/dt) and negative (–dP/dt) first time derivatives (mmHg/s).

Isolated Papillary Muscle Mechanics

Immediately after measurement of hemodynamics, hearts were removed and placed in oxygenated Krebs solution (all mmol/L: NaCl, 132; KCl, 4.69; $CaCl_2$, 1.5; $MgSO_4$, 1.16; KH_2PO_4, 1.18; $C_6H_{12}O_6$, 5.50; HEPES, 20; pH, 7.40 ± 0.02;28°). As described in our previous reports (Bocalini et al., 2010, 2014), the posterior papillary muscle was dissected carefully from the left ventricle, mounted between two spring clips, and placed vertically in a chamber containing Krebs solution oxygenated with 100% O_2. The upper portions of the muscles were attached to an isometric transducer (model FT03E; Grass Instrument, Quincy, MA, USA) connected to a micrometer for adjustment of muscle length. Preparations were stimulated 12 times/min with 5 ms square-wave pulses. The preparations were then permitted to contract isotonically under light loading conditions (0.4g) during a 60-min equilibration period. Finally, papillary muscles were loaded to contract isometrically for 15 min and stretched to the apices of their length-tension curves (L_{max}). The following parameters were measured during isometric contractions: peak developed tension (DT), resting tension (RT), maximum rate of tension development (+dT/dt), and tension decline (–dT/dt). The mechanical behavior of the papillary muscles was evaluated under basal conditions and at 92, 94, 96, 98, and 100% of L_{max}, allowing Frank-Starling curves to be determined, as previously described (Bocalini et al., 2010). Thereafter, muscle length at L_{max} was measured, and the muscle between the two clips was blotted dry and weighed. Muscle cross-sectional area (CSA) was calculated from muscle weight and length, assuming cylindrical uniformity and a muscle density value of one.

Biometric Analysis

Wet and dry weights of the right lung were obtained, and the lung water content (%H_2O) was determined as follows:

$$\%H_2O = (\mathrm{wetweight} - \mathrm{dryweight}/\mathrm{wetweight}) \times 100$$

Myocardial hypertrophy was estimated by dividing the heart weight by the body weight and defining the cardiomyocytes nuclear volumes of each rat, as is described in histomorphometric analysis.

Histomorphometric Analysis

Sections of paraffin embedded tissue (4 μm) from the remnant myocardium were stained with picrosirius red to determine collagen content using an Aperio ScanScope Console v.8.0.0.1058 (Aperio Technologies, Leica Byosistem Inc, Los Angeles, CA, USA) and a published algorithm (Samuel, 2009). Perivascular fibrosis, defined as areas of stained intramural coronary arteries, were disregarded.

Hematoxylin-eosin staining was used for defining nuclear volume. The average nuclear volume (NV) was determined randomly in 50–70 myocytes cut longitudinally for each

animal, and calculated according to the following equation (Gerdes et al., 1994):

$$NV = \pi \times D \times d^2/6$$

where D = longer nuclear diameter and d = shorter nuclear diameter.

Terminal Deoxynucleotidyl Transferase dUTP Nick end Labeling (TUNEL)

The apoptosis rate in the remnant myocardium was determined using TUNEL assays (Roche Applied Science, South San Francisco, CA, USA), according to the manufacturer's instructions. Six micrographs were randomly selected from each animal, and the numbers of healthy and apoptotic cardiomyocytes were counted. The percentage of apoptotic TUNEL-stained cells was calculated as a ratio of the total cell number.

Preparation of Heart Homogenates, SDS PAGE, and Immunoblotting

Rat hearts were minced with razor blades and homogenized in a Polytron PT 2100 homogenizer (Kinematica, AG, Switzerland) in ice-cold buffer (pH 7.4) containing 150 mM NaCl, 7.2 mM Na_2HPO_4, 2.8 mM NaH_2PO_4, 15 mM NaF, 50 mM $Na_4O_7P_2 \times 10H_2O$, and Halt Protease Inhibitor Cocktail (Thermo Fisher Scientific, Rockford, IL, USA). The Lowry method was used for protein quantitation. Homogenates were diluted in Laemmli buffer, boiled for 5 min, and proteins (60 μg) were separated by electrophoresis on 10% polyacrylamide mini-gels before electro-transfer to polyvinylidene difluoride (PVDF) membranes. Membranes were blocked with 5% skimmed milk for 2 h at room temperature and probed with primary antibodies, including anti-sodium/calcium exchanger (NCX) (1:1,000; Merck, Darstadt, Germany), anti-SERCA2 (1:1,000; Merck), anti-phospholamban (PLB; total) (1:500; Abcam, Santa Cruz Biotechnology, CA, USA), antibodies specific for PLB phosphorylated on Ser16 (1:500,000; Abcam, Santa Cruz Biotechnology, CA, USA) and Thr17 (1:100,000; Abcam, Santa Cruz Biotechnology, CA, USA), anti-mouse ryanodine receptor (1:5,000; Abcam, Invitrogen, San Diego, CA, USA), anti-caspase3 (1:500; Sigma-Aldrich, Invitrogen, San Diego, CA, USA), and anti-GAPDH (1:10,000; Abcam, Cambridge, USA), diluted in TBS-T buffer [50 mM Tris–HCl, 154 mM NaCl (pH 7.5), 0.1% Tween 20] plus 2% skimmed milk. After a 12 h incubation at room temperature, membranes were washed in TBS-T three times for 10 min and then incubated for 2 h with HRP-conjugated secondary antibodies (Invitrogen, CA, USA) diluted 1:1,000 in TBS-T plus 2% skim milk (Vector Laboratories, Los Angeles, CA, USA). Bound antibodies were detected using enhanced chemiluminescence reagent for 1 min. Bands were visualized and digitized using the ImageScanner LAS4000 mini (GE HealthCare, Little Chalfont, UK) and quantified using ImageJ software (Bethesda, MD, USA).

Statistical Analyses

Data are presented as mean ± standard error of the mean (SEM). The Kolmogorov–Smirnov test was used to estimate Gaussian distributions. Levine's test was used to characterize the homogeneity of variances. Kaplan-Meier curves were used to define mortality. Two-way ANOVA and Bonferroni tests were applied to parametric data. Kruskal-Wallis analysis with associated Dunn's test was performed for non-parametric data. Analyses were performed using SPSS 12.0 (Systat Software Inc., Richmond, CA, USA) and GraphPad Prism 5.0 (GraphPad Software Inc., San Diego, CA, USA), and $p < 0.05$ was considered significant.

RESULTS

The reperfusion procedure was unsuccessful in 47 (30%) of the 156 animals subjected to temporary coronary occlusion.

Mortality

Kaplan-Meier analysis demonstrated that the survival of rats exercised prior to coronary occlusion was prolonged compared to sedentary rats (**Figure 1**). Expressive mortality occurred during 24 h following coronary occlusion. As reported in the literature (Neri et al., 2017), myocardial reperfusion frequently promotes arrhythmias, that can explain lower survival in this group. After 10 days of MI, survival curves were parallels. Differences in early mortality resulted in mortality differences between groups. Four weeks after coronary occlusion, the survival rates of rats in the EPO (11/12; 92%) and EDR (19/22; 86%) groups were higher than those of rats in the SPO (32/50; 64%) and SDR (14/28; 50%) groups. In addition, SPO survival was higher than SDR. However, survival was not significantly different between the two groups of exercised rats: EPO and EDR.

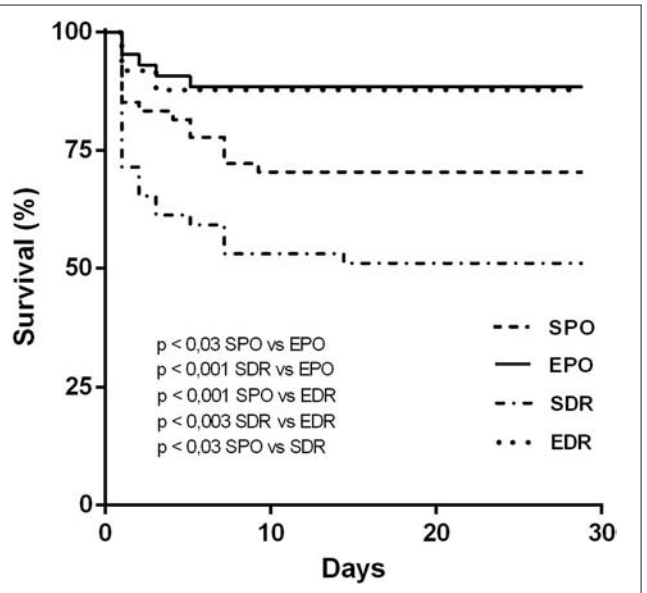

FIGURE 1 | Kaplan–Meier curves illustrating the probability of survival vs. time in animals from sedentary permanent occlusion (SPO), exercised permanent occlusion (EPO), sedentary delayed reperfusion (SDR), and exercised delayed reperfusion (EDR) groups. Differences were evaluated using the log-rank (Mantel-Cox) test.

TABLE 1 | Cardiac parameters (mean ± SEM) of exercised and sedentary rats subjected to MI with or without LR.

	SPO ($n = 12$)	EPO ($n = 12$)	SDR ($n = 8$)	EDR ($n = 9$)
MI size (%)	44 ± 1	47 ± 2	46 ± 1	49 ± 2
DA (mm^2)	0.59 ± 0.03	0.61 ± 0.02	0.63 ± 0.06	0.54 ± 0.02
SA (mm^2)	0.54 ± 0.03	0.43 ± 0.03	0.42 ± 0.06	0.40 ± 0.03
SF (%)	30 ± 0.02	30 ± 0.03	35 ± 0.04	31 ± 0.02
E/A ratio	5 ± 0.5	5.8 ± 0.24	5.2 ± 0.6	4.4 ± 0.6
LVSP (mmHg)	98 ± 4	112 ± 5*	119 ± 5*	113 ± 5*
LVDP (mmHg)	22 ± 2	24 ± 1	20 ± 1	15 ± 1#
+dP/dt (mmHg/s)	5,052 ± 368	5,453 ± 198	6,719 ± 594*#	6,856 ± 121*#
–dP/dt (mmHg/s)	3,165 ± 243	3,833 ± 156*	4,023 ± 190*	4,290 ± 488*
LV weight (mg)	0.69 ± 0.01	0.58 ± 0.03	0.65 ± 0.04	0.63 ± 0.01
RV weight (mg)	0.20 ± 0.02	0.21 ± 0.01	0.23 ± 0.02	0.19 ± 0.01
$\%H_2O$	80 ± 0.4	79 ± 0.1	80 ± 0.5	78 ± 0.2*&
NV (μm^3)	309 ± 15	203 ± 17*	257 ± 24	182 ± 16*&
Collagen (%)	6.29 ± 1.08	6.31 ± 1.29	2.27 ± 0.44*#	2.41 ± 0.38*#

*SPO, sedentary permanent occlusion; EPO, exercised permanent occlusion; SDR, sedentary delayed reperfusion; EDR, exercised delayed reperfusion; MI size, infarct size; DA, diastolic left ventricular area; SA, systolic left ventricular area; LV weight, left ventricle weight; RV weight, right ventricle weight; LVSP, left ventricular systolic pressure; LVDP, left ventricular diastolic pressure; +dP/dt, positive first time derivate; –dP/dt, negative first time derivate; $\%H_2O$, lung water content; NV, nuclear volume; Collagen, collagen content; SF, shortening fraction E/A ratio. Differences were evaluated by two-way ANOVA followed by post-hoc Bonferroni test. *$p < 0.05$ vs. SPO; #$p < 0.05$ vs. EPO; &$p < 0.05$ vs. SDR.*

MI Size, Pulmonary Congestion, and Biometrics

MI size, pulmonary water content, and biometrics are detailed in **Table 1**. There were no statistically significant differences in infarct size among the four groups. Lung water content, a measure of pulmonary congestion, was significantly lower in the EDR group compared with the sedentary groups (SPO and SDR), whereas the EPO group did not differ from the other groups. To evaluate myocardial hypertrophy, the average myocardial mass and nuclear volume from each group were assessed. Myocardial mass did not differ significantly among the groups, but nuclear volume was significantly lower in the exercised groups (EPO, EDR) than in sedentary animals (SPO, SDR).

ECHO and Hemodynamics

No differences in diastolic and systolic area, shortening fraction, or E/A ratio were observed between the groups by ECHO (**Table 1**).

The LVSP of the SPO group was lower than those of the other groups (**Table 1**), whereas the LVDP of the EDR group was lower than that of the EPO group. There were no other differences in LVDP values among the groups. Notably, DR enabled higher values of +dP/dt than those of animals subjected to permanent occlusion.

Mechanical Response of Papillary Muscles

Posterior papillary muscles were isolated to examine their mechanical behavior in response to exercise and permanent or temporary coronary occlusion. There were higher DT in both reperfused group of rats than in rats subjected to permanent occlusion (**Figure 2**). Values of +dT/dt were elevated in EDR rats compared to those in the other groups, whereas, in respect to –dT/dt, there was no significant difference among groups. There were also no significant differences among RT of the four groups. In addition, the length- active tension ratios, which are indicative of the Frank-Starling mechanism, were steeper in the DR groups than the permanent occlusion groups. Altogether, the mechanical behavior exhibited by posterior papillary muscles suggests that muscle contraction is enhanced in DR animals.

Collagen and Apoptosis in the Remaining Myocardium

Collagen levels (**Table 1**) were significantly lower in the reperfused rats than those of the SPO and EPO groups. Moreover, apoptosis was significantly reduced in the EDR and SDR groups compared to the permanent occlusion groups, indicated by reduction of TUNEL positive cells (**Figure 3A**) and decreased cleaved caspase 3 (**Figure 3B**).

Myocardium Protein Levels

There were no significant differences among groups in respect to calcium kinetics proteins (**Figure 3**): sodium/calcium exchanger (Panel C), SERCA 2 (Panel D), total PLB (Panel E), and p-PLB (Panel F).

DISCUSSION

Myocardial necrosis following coronary occlusion poses a serious risk to human lifes and motivates reperfusion. When not possible early reperfusion, late reperfusion should be considered. Late reperfusion has been shown, in some recent studies, to benefit ischemic symptomatic patients reperfused more than 12 h after the onset of myocardial infarction (Ibanez et al., 2018; Nepper-Christensen et al., 2018; Yang et al., 2018). In infarcted patients without symptoms of residual myocardial ischemia there is no clear evidence of late reperfusion benefits. We analyzed the model of delayed reperfusion in sedentary and exercised rats for examination of mortality, cardiac

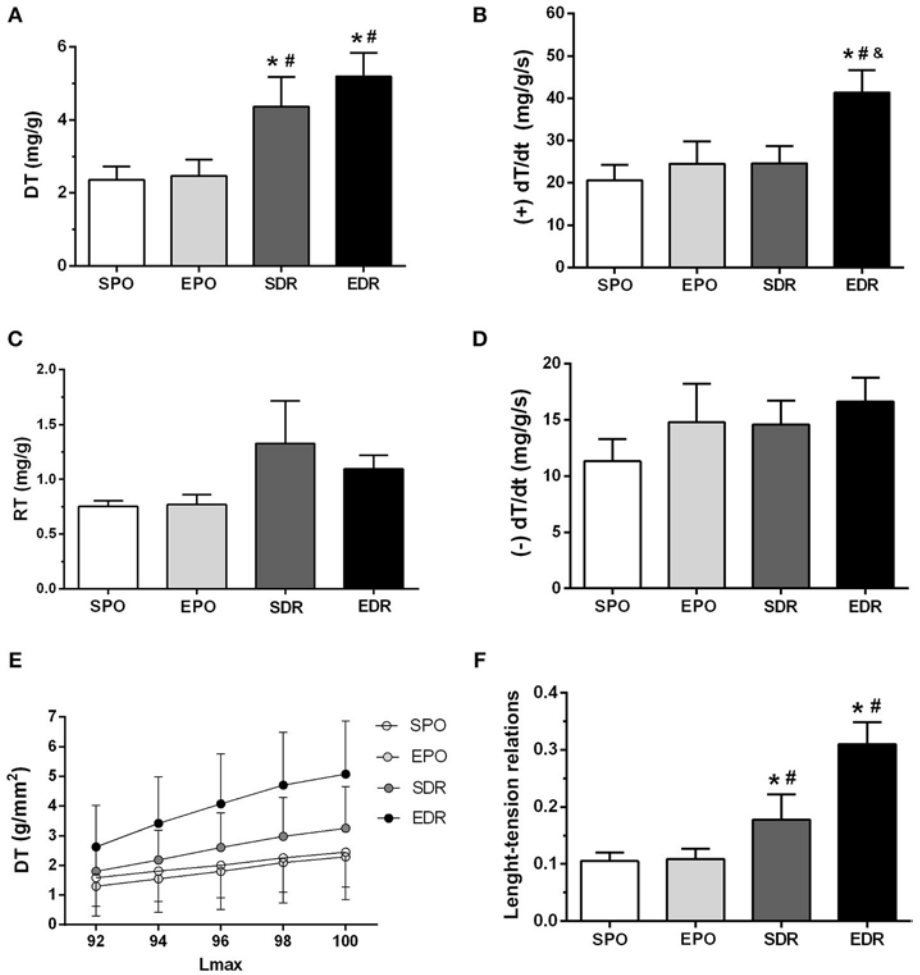

FIGURE 2 | Papillary muscle parameters. **(A)** DT, developed tension. **(B)** +dT/dt, maximum positive derivative. **(C)** RT, resting tension. **(D)** –dT/dt, maximum negative derivative. **(E)** Plots of DT (g/mm^2 × %Lmax). **(F)** Length-active tension relations slopes. Differences were evaluated by two-way ANOVA followed by *post-hoc* Bonferroni test. $^{*}p < 0.05$ vs. SPO; $^{\#}p < 0.05$ vs. EPO; $^{\&}p < 0.05$ vs. SDR.

remodeling, function, histology and molecular composition of remnant myocardium.

Our data showing comparable myocardium infarction sizes in permanent and temporarily coronary occluded rats indicate that 1 h of coronary occlusion successfully promotes total necrosis of risk areas in the rat heart. These results are in accordance with previous reports (Hedstrom et al., 2009) and support our previous findings (dos Santos et al., 2009).

Previous papers of Nakagawa et al. (2008) and Takemura et al. (2009) reported benefits in late reperfused compared to those with permanent occlusion; there is no notice about mortality. Our results related to mortality are comparable to several human studies that indicated no benefits on survival for patients who received coronary late reperfusion (Moreno et al., 1994; Horie et al., 1998; Yousef and Marber, 2000). Nevertheless, our Kaplan- Meier analysis demonstrated that previous swimming prolonged the survival of rats following temporary and permanent occlusion. Exercise prior to infarction has been reported to decrease mortality of infarcted rodents (Dayan et al., 2005; De Waard and Duncker, 2009).

In the current study, there were no statistically significant differences in cardiac mass of the right and left ventricles between groups, consistent with our previous findings (Veiga et al., 2011, 2013). However, nuclear volume, a more appropriate index of myocardial hypertrophy (Mill et al., 2011; Bajgelmen et al., 2015), indicated attenuation of hypertrophy in the exercised groups compared with the sedentary groups. To our knowledge, no previous studies have analyzed nuclear volume using a similar experimental protocol.

Although ECHO did not identify any difference among groups, there were indications of improved ventricular performance and remnant myocardial inotropism in reperfused animals. For example, LVSP, +dP/dt, and papillary muscle DT were improved after DR. Moreover, exercise intensified remote myocardial advantages following DR. In fact, LV end diastolic

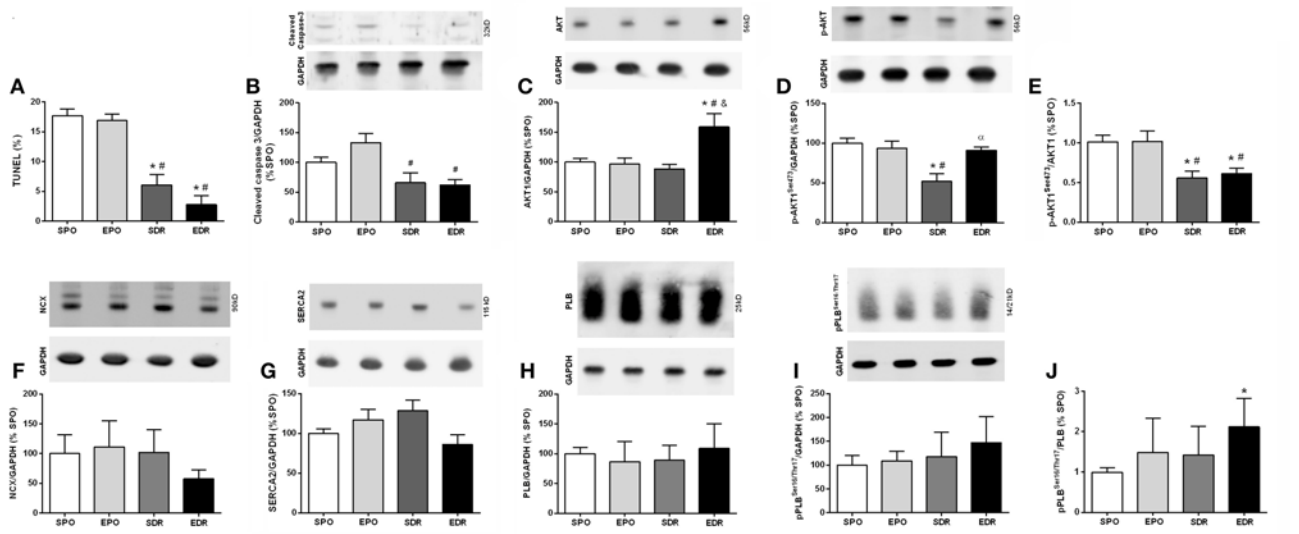

FIGURE 3 | Apoptosis and calcium kinetics in sedentary permanent occlusion (SPO), exercised permanent occlusion (EPO), sedentary delayed reperfusion (SDR), and exercised delayed reperfusion (EDR) groups. **(A)** Percentage of apoptotic cells by TUNEL analysis. **(B)** Cleaved caspase-3. **(C)** AKT1. **(D)** Phosphorylated AKT1 (pAKT1). **(E)** Ratio of pAKT1/AKT1. **(F)** Sodium calcium exchanger (NCX). **(G)** SERCA2. **(H)** Phospholamban (PLB). **(I)** Phosphorylated PLB (pPLB) at serine (ser) 16 (PLB ser16) and threonine (thr) 17 (PLB thr 17). **(J)** Ratio of pPLB/PLB. Differences were evaluated by two-way ANOVA followed by *post-hoc* Bonferroni test. $^{*}p < 0.05$ vs. SPO; $^{\#}p < 0.05$ vs. EPO; $^{\&}p < 0.05$ vs. SDR; $^{\alpha}p < 0.05$ vs. EDR.

pressure, pulmonary water content, nuclear volume and the papillary length-active tension relationship slope were ameliorated after exercise in DR animals. Remarkably, exercise did not result in functional benefits in rats with permanent occlusion.

Although our calcium kinetics proteins data showed no relevant differences among groups, maximum DTs were higher in reperfused rats, and length-active tension relationships disclosed a Frank-Starling mechanism enhancement. Overall, these results suggest that calcium sensitivity may be improved in myofilaments.

Finally, our histologic analysis revealed meaningful benefits of DR in the remote myocardium. Indeed, reduced cleaved caspase-3 and TUNEL staining are in accord with lower levels of apoptosis in DR groups. These results are unprecedented for late myocardial reperfusion. Moreover, the clear reduction in collagen content in the remote myocardium of rats subjected to DR relative to those in non-reperfused animal's highlights additional benefits of DR. There is one unique previous work analyzing collagen content after late reperfusion (Nakagawa et al., 2008), reporting reduction of collagen in late reperfused rats.

Conclusion

The main findings of this study were: (1) clear signs of remodeling with pathological hypertrophy, systolic and diastolic cardiac dysfunction in animals subjected to permanent occlusion; (2) these parameters were minimized after DR; (3) great remote myocardial benefits in delayed reperfused animals, including inotropism enhancement, pulmonary congestion reduction, and attenuation of collagen, apoptosis; (4) prolonged survival in rats exercised prior to coronary occlusion.

Clinical Implications and Study Limitations

As in all translational studies, certain limitations to translate the finding to clinical use should be applied. Occlusion of coronary artery due to plaque rupture is an entirely different situation than artery ligation in animals. In the clinical scenario, drugs are used, background of cardiovascular risk factors and age may differentiate MI evolution. As no human cellular data after late reperfusion is available for comparison with our results, it is not possible to directly translate to medical practice our data obtained using rats. However, our unprecedented findings recommend that the clinical practice should consider the effectiveness of coronary reperfusion in humans, when the time to complete necrosis is borderline.

AUTHOR CONTRIBUTIONS

EV: conception and design of the study, acquisition of data, drafting the article and revising it critically for important intellectual content, and final approval of the version to be submitted. EA, DB, CP, AAS, FM, RL, and BL: acquisition of data, interpretation of data, and final approval of the version to be submitted. AG: introduction of a new technique, drafting the article and revising it critically for important intellectual content, and final approval of the version to be submitted. AJS: drafting the article and revising it critically for important intellectual content and final approval of the version to be submitted. PT: conception and design of the study, drafting the article and revising it critically for important intellectual content, and final approval of the version to be submitted.

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Effects of Acute Aerobic Exercise on Cognition and Constructs of Decision-Making in Adults With and Without Hypertension

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Hypertension accelerates brain aging, resulting in cognitive dysfunction with advancing age. Exercise is widely recommended for adults with hypertension to attenuate cognitive dysfunction. Whether acute exercise benefits cognitive function in this at-risk population is unknown. The purpose of this study was to compare the effects of acute aerobic exercise on cognitive function in 30 middle-aged hypertensive (HTN) and 30 age, sex, and body mass index (BMI)-matched non-HTN adults (56 ± 6 years, BMI 28.2 ± 2.9 kg/m^2; 32 men). Subjects underwent cognitive testing pre/post 30-min cycling (≈55% peak oxygen consumption). Cognition was assessed using standard metrics of accuracy and reaction time (RT) across memory recognition, 2-back, and Flanker tasks. Behavioral data was further analyzed using drift-diffusion modeling to examine underlying components of decision-making (strength of evidence, caution, bias) and RT (non-decision time). Exercise elicited similar changes in cognitive function in both HTN and non-HTN groups ($p > 0.05$). Accuracy was unaltered for Flanker and 2-back tasks, while hits and false alarms increased for memory recognition post-exercise ($p < 0.05$). Modeling results indicated changes in memory hits/false alarms were due to significant changes in stimulus bias post-exercise. RT decreased for Flanker and memory recognition tasks and was driven by reductions in post-exercise non-decision time ($p < 0.05$). Our data indicate acute exercise resulted in similar, beneficial cognitive responses in both middle-age HTN and non-HTN adults, marked by unaltered task accuracy, and accelerated RT post-exercise. Additionally, drift-diffusion modeling revealed that beneficial acceleration of cognitive processing post-exercise (RT) is driven by changes in non-decision components (encoding/motor response) rather than the decision-making process itself.

Keywords: acute exercise, hypertension, executive function, mathematical modeling, memory, short-term, cognition, decision making

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INTRODUCTION

Cognitive function is one of the most important determinants of health, function, and quality of life with advancing age (Wilson et al., 2013). Hypertension accelerates brain aging (Gasecki et al., 2013) and impairs cognitive function (Elias et al., 1993; Shehab and Abdulle, 2011; Iadecola et al., 2016). The cognitive domains of executive function [high-level interrelated cognitive abilities that

integrate lower-level functions to complete goal-directed behavior (Logue and Gould, 2014)] and memory appear to be most vulnerable to hypertension (Shehab and Abdulle, 2011; Hughes and Sink, 2015). Identifying lifestyle modifications that can improve cognitive function in these domains is therefore of particular interest for adults with hypertension.

Aerobic exercise is highly recommended (Pescatello et al., 2004; Brook et al., 2013) for adults with hypertension to not only help control blood pressure but to maintain cognitive health (Gorelick et al., 2011) and prevent cognitive impairment with advancing age (Pedersen and Saltin, 2006; Lange-Asschenfeldt and Kojda, 2008). Despite these recommendations there is a paucity of data on the effect of exercise on cognitive function in adults *with hypertension*. Indeed, only 1 study has investigated the effects of exercise training on cognitive function in hypertension (noting no significant effects) (Teixeira et al., 2015). Previous studies have suggested the acute cognitive response to exercise may offer insight into origins of neuro-cognitive training adaptations (Basso and Suzuki, 2017). Cognitive responses to acute exercise may additionally provide insight into underlying neural plasticity by revealing whether exercise induces acute changes in cognitive function (Ludyga et al., 2016). Hypertension is linked to diffuse structural brain damage/atrophy (Gasecki et al., 2013) which may disrupt neural plasticity and alter the ability of the brain to respond to exercise. Currently, however, no data exists on acute cognitive responses to exercise in hypertension.

Meta-analytical investigations in non-hypertensive populations have shown that acute exercise improves executive function (Tomporowski, 2003; Chang et al., 2012; McMorris and Hale, 2012) and may positively impact memory (Tomporowski, 2003; Chang et al., 2012). Moderate to vigorous exercise appears to produce the most pronounced, beneficial changes in both executive function, and memory (Tsukamoto et al., 2017). Whether hypertensives experience similar improvements in executive function and memory performance as their non-hypertensive counterparts following a bout of exercise is unknown.

When studying the effects of exercise on cognitive function, traditional analytical approaches rely on reverse inference to survey accuracy or processing speed/reaction time (RT). Greater accuracy is believed to reflect better functioning of the cognitive domain of interest (i.e., executive function, memory). Faster RT is also posited to indicate improved functioning in the cognitive domain of interest since less time is required for the individual to respond correctly to the stimulus. Reverse inference is valid only if the changes in behavior (in this example RT) are driven solely by the cognitive domain being surveyed. There is substantial evidence that factors related to the decision making process impact both RT and accuracy separate from any direct impact on the cognitive domain of interest (White et al., 2016).

Drift-diffusion modeling (DDM) is a mathematical approach (Forstmann and Wagenmakers, 2015) that decomposes observational data into latent processes underlying decision-making. Constructs elucidated by DDM include caution, encoding, motor response duration, strength, and quality of evidence presented by the stimulus, and bias (i.e., implicit or explicit preference for one response over another) (White et al., 2011, 2016; White and Poldrack, 2014). Thus, DDM attempts to describe changes in the latent decision-making process that are responsible for the observed responses. DDM incorporates *all available behavioral data* (accuracy, correct/error RT, shape of RT distributions) rather than solely relying on RT for correct trials and accuracy to describe changes in behavior. This modeling technique can provide novel insight into whether changes in cognitive function stemming from acute exercise (observed through accuracy and RT) are due to neural (i.e., encoding, motor response) or behavioral changes (i.e., caution, bias) in the latent constructs of decision-making.

As such, the purpose of this investigation was to examine the effect of acute aerobic exercise on cognitive function (using memory and executive function tasks) and latent constructs of decision making in hypertensive (HTN) and non-hypertensive (Non-HTN) middle-aged adults. It was hypothesized that acute exercise would differentially affect cognitive function in HTN (unaltered performance) and Non-HTN (improved performance; manifesting as improved accuracy and accelerated RT post-exercise on executive function and memory tasks).

MATERIALS AND METHODS

Participants

Thirty middle-aged HTN (56 $\pm$ 6 years; 14 women) and 30 age-, sex-, and body mass index (BMI)-matched non-HTN adults (56 $\pm$ 6 years; 14 women) were recruited for this study. This investigation was part of a larger study designed to investigate the vascular and cognitive responses to acute exercise in hypertension. While the vascular responses are published elsewhere (Lefferts et al., 2018b) the current paper will present the cognitive results. We targeted middle-aged adults because (1) cognitive decline can be detected as early as middle-age (Singh-Manoux et al., 2012), making this age range a prime target for preventive research and (2) recent meta-analyses indicate this is an understudied group regarding acute exercise and cognitive function (Ludyga et al., 2016). Exclusion criteria included self-reported smoking, stroke, dementia, diabetes mellitus, severe obesity (BMI $\geq$ 35 kg/m^2), previous cardiovascular events, pulmonary/renal/neurological disease, or recent head trauma (concussion). Participants were free from dementia (Montreal Cognitive assessment score $\leq$ 21), and depression [assessed using the center for epidemiologic studies depression (CESD) questionnaire]. Hyperlipidemic and overweight participants (BMI 25–30 kg/m^2), were included in the sample due to the high prevalence of these risk factors within middle-aged adults (regardless of HTN status). Menopausal status (pre-, peri-, post-menopausal) was assessed according to STRAW+10 guidelines. This study was carried out in accordance with the recommendations of Syracuse University Institutional Review Board. The protocol was approved by the Syracuse University Institutional Review Board. All subjects gave written informed consent in accordance with the Declaration of Helsinki prior to study initiation.

All HTN participants were diagnosed and undergoing treatment for hypertension. Participants did not refrain from HTN medication during testing due to concern of rebound hypertension, and to improve external validity, as the medicated state is the "natural state" in which the majority of HTN would engage in exercise. All participants underwent descriptive testing (fasting lipid/glucose assessment, anthropometrics, body composition, VO_2peak assessment during an incremental cycling protocol to volitional fatigue) and familiarization prior to the acute exercise visit. For more information on these methods, the reader is directed to the following reference (Lefferts et al., 2018b).

Study Design

Participants were instructed to arrive >4-h fasted, and abstain from non-essential medication, caffeine, alcohol, and exercise the day of the visit. All acute exercise visits were standardized to the morning. Participants underwent cognitive measures pre and post a 30-min bout of aerobic exercise (**Figure 1**). Pre-exercise cognitive testing occurred following 15-min of supine rest. Post-exercise measures were assessed ∼10–12 min post because cognitive function may be negatively affected within the first 10 min post exercise (Chang et al., 2012). All pre and post measures were assessed in the supine position.

Acute Exercise Protocol

The acute aerobic exercise bout was 30-min of moderate-intensity cycling (57.1 ± 3.5% VO_2peak). This exercise dose/intensity is recommended by the American Heart Association and American College of Sports Medicine for adults <65 years of age (Haskell et al., 2007), and elicits positive effects on cognitive function post-exercise (Chang et al., 2012) in healthy adults. Exercise intensity was confirmed and titrated by measuring oxygen consumption during two separate periods of the exercise bout (min 5–10, and 20–25).

Cognitive Measures

Cognitive function was assessed using a 15-min, 3-task computerized (Matlab, The MathWorks, Natick, MA; and PsychToolbox) cognitive battery (with a hand-held response clicker) that interrogates memory (word recognition task) and the attention (Flanker task), and working memory (2-back version of an n-back task) components of executive function. This battery of tasks has been used by our group previously (Lefferts et al., 2016). Prior to starting the current study, we tested the within- (tests separated by 5-min break) and between-day (24-h between tests) reliability of our cognitive tasks in 19 healthy adults (age range 18–35 yr; 8 women). This data indicated moderate to strong within-day reliability (ICC's 0.62–0.80) for cognitive metrics (hits/reaction time) for memory recognition and Flanker tasks (see **Supplemental Table S1**). Within-day reliability of the 2-back was low from this preliminary study, so 2-back task rules were adjusted prior to conducting the current study such that participants were required to respond to non-match items (rather than solely match items). Literature suggests the memory recognition paradigm and flanker task are valid and reliable measures of memory and attention, respectively (Fuchs et al., 1999; Zelazo et al., 2014). While there are mixed findings regarding the validity of the 2-back as a measure of working memory, it is reliable (Jacola et al., 2014) and helpful in identifying differences in cognitive processing between groups/clinical populations (Miller et al., 2009), and following acute exercise (Basso and Suzuki, 2017). These particular domains were selected because they have implications for later-life cognitive function and are affected by HTN and acute exercise (Chang et al., 2012). The battery always began with the word study list, followed by the working memory and executive function tasks in a randomized, counter-balanced order, followed by the memory recognition task. Each trial was preceded by verbal instructions and a visual reminder of each task and its respective goals, in addition to brief on-screen instructions prior to beginning each task once testing had begun. This multi-stage instruction process was designed to ensure participants recalled the goal of each task prior to initiation.

Participants were familiarized with all cognitive tasks prior to the acute exercise visit to account for learning effects. Familiarization included point-by-point written and verbal instructions for each task, followed by a complete practice session of the cognitive battery. If participants did not adequately understand a task they were permitted to repeat the task until they were comfortable with the goals and procedures of the task.

Memory

Memory recognition was assessed by presenting participants with 36 words for memorization and later recognition from memory. The list contained 36 concrete words from the English language that were displayed for 1 s each. Participants then completed two cognitive tasks (flanker and 2-back, randomized order) before beginning the memory recognition portion of the test (∼10 min later). To assess memory recognition performance, participants were presented with 72 words (36 distracters), at a rate of 1 every 2 s, and instructed to identify the words as "old" if they remembered the word from the study list or "new" if the word being presented was not on the study list. Accuracy was expressed as percent hits (correctly recalled items) and false alarms (old/studied items incorrectly identified as new/distractors). Mean reaction time (RT) for hits was calculated to assess processing speed.

Executive Function

The attention component of executive function was assessed using the Eriksen Flanker task. Participants were presented with 5 arrows (standard Eriksen Flanker task) and instructed to respond to which direction the middle arrow was pointing. This task contained 64 congruent (i.e., all arrows facing the same direction; <<<<<) and 64 incongruent (i.e., flanking arrows facing different direction from middle arrow; <<><<) 1.5-s long trials (totaling ∼5-min). The 128 items were accumulated through 2 consecutive runs of 64 congruent/incongruent items, separated by a 10-s break. Accuracy was expressed as percent hits and calculated as hits / (hits + incorrect), where missed stimuli (i.e., no response within the 1.5 s window) were ignored and not counted as incorrect. Processing speed was assessed as mean hit RT for hit incongruent/congruent trials.

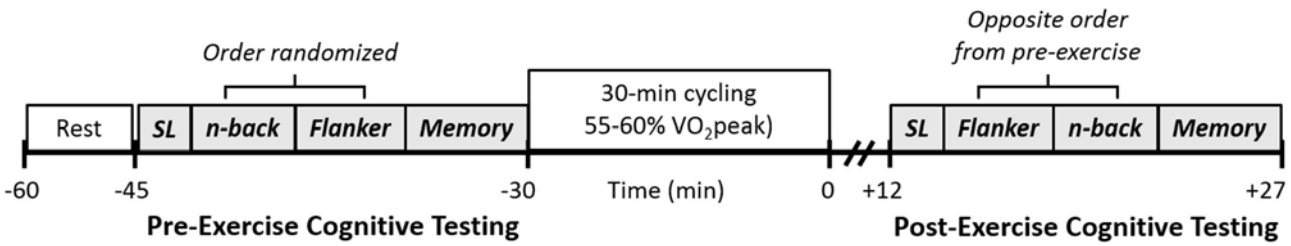

FIGURE 1 | Study design for assessing cognitive function pre and post moderate intensity exercise aerobic exercise. SL, study list.

The working memory component of executive function was assessed using a 2-back number task. This task included 160 trials divided into 2 runs of 80 items, separated by a 10-s break. The task lasted ∼4 min. After fixating on 3 crosses, participants were presented with a series of digits (1–9) at a rate of 1/s, with consecutive numbers separated by 0.25 s. They were instructed to press the right response button if the number presented matched the number that was presented 2 numbers before (i.e., 4-7-4) and press the left response button for all non-match stimuli. Accuracy was expressed as percent hits (hits/(hits + errors)) and commission errors (falsely identifying a number as a match), and processing speed was assessed as mean hit RT.

Drift-Diffusion Modeling

Drift-diffusion modeling (DDM) was conducted *post-hoc* on all cognitive performance data. This modeling technique can provide insight into whether changes in cognitive function are due to neurological (i.e., encoding) or behavioral (i.e., caution, bias) changes. DDM has been validated (Voss et al., 2004) and described in detail previously (White et al., 2011; White and Poldrack, 2014). In short, the model assumes that decisions start at a point (z) and noisy evidence is sampled until a boundary (a/0) is reached, initiating a response. Wider distances between boundaries (a) indicate slower but more accurate responses (caution). Drift rate (v) indicates the strength of evidence (higher drift rate means stronger evidence), and non-decision time estimates the duration of encoding/motor response. DDM parameters are visually represented and summarized in our previous work (Lefferts et al., 2016).

Statistical Analyses

No studies to date have examined the acute effects of exercise on cognitive function adults with HTN compared to Non-HTN, making it difficult to directly power this study to detect group-by-time interactions. Previous pilot data in young adults indicated changes in RT had an effect size (Cohen's d) of 0.82 for reductions in post-exercise RT. *A priori* power calculations (2-tailed t-test, independent groups) using the effect size of 0.82, a power of 0.80, and α of 0.05 indicated 25 participants per group would be sufficient to detect changes in cognitive function post-exercise. This sample size is similar to studies examining effects of blood pressure on cognitive function (≈25 per group) (Shehab and Abdulle, 2011). As such, we collected data on 30 HTN and 30 Non-HTN adults.

All data is reported as mean ± standard deviation and statistical significance was established *a priori* as $p < 0.05$. Data normality was assessed quantitatively using the Shapiro-Wilk test. Between-day and within-day reliability were analyzed using intraclass correlation coefficients from the cognitive familiarization trial and pre-exercise cognitive testing (run 1 vs. run 2 of executive function tasks). Non-normally distributed RT and DDM metrics were transformed to meet normality assumptions. Descriptive characteristics were compared using independent T-tests for continuous variables and χ^2 tests for categorical data. We examined cognitive RT and DDM parameters in non-HTN vs. HTN groups across pre- and post-exercise time points using a 2 x 2 [2 group x 2 time] repeated measures ANOVA. If a significant group x time interaction was detected, it was further explored using Bonferroni corrected *post-hoc* tests. All accuracy metrics (hit rates) were unable to be successfully transformed to meet assumptions. Accuracy metrics were therefore analyzed using Mann-Whitney U-tests to test the effect of group (HTN vs. Non-HTN), and group by time interaction (change in accuracy post-pre for HTN vs. Non-HTN), with Wilcoxon signed-rank tests used to test the effect of time (pre- vs. post-exercise). All significant non-parametric analyses were corrected for multiple comparisons via Bonferroni correction since these analyses could not be run simultaneously. Effect sizes are presented with their corresponding p-values and were calculated as Cohen's D, partial eta squared (η^2), and r^2 (Z^2/n) for descriptive characteristics, ANOVA, and non-parametric analyses, respectively.

RESULTS

Cognitive Task Reliability

Intraclass correlation coefficients for between-day reliability were statistically significant and ranged from moderate to strong across all cognitive tasks (**Supplemental Table S1**). Pertinent to this study design, we noted moderate to strong within-day reliability for the pre-exercise Flanker and 2-back tasks (intraclass correlation coefficients 0.72–0.92, $p < 0.05$).

Group Characteristics and Acute Exercise Intensity

As described elsewhere (Lefferts et al., 2018b), groups were well-matched for descriptive characteristics (including sex, age, body size, body composition, fasting lipids, depression

symptomology, and menstrual status; **Table 1**). Fasting glucose was significantly higher in HTN vs. Non-HTN ($p < 0.05$). Cardiorespiratory fitness and accumulated minutes of moderate-to-vigorous physical activity ($p < 0.05$) were significantly higher, and average number of steps over 6 d tended to be higher in Non-HTN ($p = 0.06$) compared to HTN. Statin use was greater in HTN vs. Non-HTN ($p < 0.05$). On average, HTN participants had been diagnosed for 129 ± 97 months. HTN participants took their blood pressure medication at similar times of day (63.3%, AM vs. 33.0%, PM; $p = 0.095$), with one participant taking medication at both times of day. At-home systolic and diastolic blood pressure were higher in HTN (systolic 126 ± 12 mmHg, diastolic 79 ± 8 mmHg) than Non-HTN (systolic 116 ± 9 mmHg, diastolic 73 ± 6 mmHg; $p < 0.05$).

Acute Exercise

There were no differences in aerobic exercise intensity between HTN and Non-HTN for %relative VO_2peak (Non-HTN, 57.6 ± 3.6% vs. HTN, 56.5 ± 3.5% $p > 0.05$) and %maximal heart rate (Non-HTN, 69.7 ± 5.5 vs. HTN, 71.4 ± 7.3 $p > 0.05$). Absolute workload during exercise was higher for Non-HTN vs. HTN (Non-HTN, 82 ± 43W vs. HTN, 63 ± 22W $p < 0.05$).

Post-exercise Testing

Participants began supine rest within 27 ± 6 and 26 ± 6 s following cessation from exercise for Non-HTN and HTN groups, respectively ($p > 0.05$). Post-exercise cognitive testing was initiated at similar times between groups (memory study list, Non-HTN, 12.12 ± 1.25 min vs. HTN, 11.96 ± 1.10; Flanker, Non-HTN, 16.14 ± 2.67 min vs. HTN, 16.78 ± 4.00 min; 2-back, Non-HTN, 17.05 ± 3.26 min vs. HTN 16.74 ± 3.4 min; Memory, Non-HTN, 24.27 ± 1.23 min vs. HTN 23.57 ± 2.34 min; $p > 0.05$). The higher variability for post-exercise timing of Flanker and 2-back is related to the randomized, counter-balance design.

Effect of Exercise on Accuracy and RT

Hand-held clicker malfunctions resulted in lost data for 2 non-HTN individuals on the Flanker task and 1 non-HTN individual on the 2-back. Thus, data are presented for $n = 28$ and 29 for Flanker and 2-back, respectively, among Non-HTN. No significant group, time, or group-by-time effects were detected for accuracy as measured by hit rates on congruent/incongruent Flanker or 2-back ($p > 0.05$; **Table 2**). A time effect was detected for memory hit rate, which increased post-exercise in both groups ($p < 0.05$; **Table 3**). This was accompanied by a time effect for false alarm rate, which also increased post-exercise ($p < 0.05$). Time effects were detected for Flanker and Memory hit RT, which decreased post-exercise ($p < 0.05$). There was a tendency for hit RT to decrease post-exercise on the 2-back although this did not reach statistical significance ($p = 0.09$). No statistical effects for discriminability were detected. No group or group by time interactions were observed at the $p < 0.05$ level, indicating that (1) there were no inherent statistical group differences in cognitive performance and (2) the statistical effect of acute exercise on accuracy and processing speed were similar between HTN and Non-HTN individuals.

TABLE 1 | Descriptive characteristics for non-HTN and HTN groups (mean ± SD unless otherwise noted).

	Non-HTN	HTN	*p*-Value (effect size)
Sex (male/female)	16/14	16/14	–
Age (years)	56 ± 6	56 ± 6	0.93 (0.00)
ANTHROPOMETRICS			
Height (cm)	169.8 ± 11.3	171.3 ± 9.6	0.57 (0.14)
Weight (kg)	82.0 ± 13.3	82.4 ± 12.5	0.91 (0.03)
Body fat (%)	32.2 ± 8.4	31.4 ± 6.9	0.69 (0.10)
Body mass index (kg/m^2)	28.3 ± 2.6	28.0 ± 3.3	0.71 (0.10)
MEDICATIONS, %(*n*)			
Statin	6.7 (2)	40.0 (12)	**0.01**
Birth control	3.3 (1)	0.0 (0)	–
Hormone replacement therapy	3.3 (1)	3.3 (1)	–
Hypothyroid	3.3 (1)	3.3 (1)	–
ACE inhibitor	–	43.3 (13)	–
ARB	–	33.3 (10)	–
Diuretic	–	50.0 (15)	–
β-Blocker	–	13.3 (4)	–
CCB	–	13.3 (4)	–
Combination therapy (≥2)	–	43.3 (13)	
LIPID PROFILE			
Hemoglobin (g/dL)	14.2 ± 0.9	13.8 ± 1.3	0.12 (0.36)
Total cholesterol (mg/dL)	202 ± 39	192 ± 36	0.28 (0.27)
HDL (mg/dL)	58 ± 17	56 ± 20	0.56 (0.11)
Triglycerides (mg/dL)	103 ± 61	116 ± 56	0.28 (0.22)
LDL (mg/dL)	128 ± 41	114 ± 30	0.17 (0.39)
Non-HDL (mg/dL)	144 ± 44	136 ± 32	0.43 (0.21)
Total cholesterol:HDL	4 ± 2	4 ± 1	0.89 (0.00)
Glucose (mg/dL)	94 ± 9	102 ± 16	**0.03 (0.62)**
QUESTIONNAIRES			
CESD	6 ± 5	7 ± 4	0.71 (0.22)
FITNESS			
VO_2peak (mL/kg/min)	32.4 ± 8.8	27.2 ± 5.6	**0.01 (0.71)**

ACE, angiotensin converting enzyme; ARB, angiotensin II receptor blocker; CCB, calcium-channel blocker; HDL, high-density lipoprotein; LDL, low-density lipoprotein; CESD, Center for Epidemiologic Studies Depression questionnaire; VO_2peak, peak oxygen consumption. Effect size calculated as Cohen's D. Bold value denotes $p < 0.05$.

Effect of Exercise on Constructs of Decision-Making

Significant time effects were detected for memory stimulus bias (increased post-exercise), and 2-back and memory non-decision time (decreased post-exercise). Similar effects were noted for the Flanker non-decision time post-exercise but this did not reach statistical significance ($p = 0.066$). No significant group or group by time interactions were detected for DDM metrics, indicating that (1) there were no underlying statistical group differences in decision making, and (2) the statistical effect of acute exercise on latent processes of decision making were similar between HTN and Non-HTN groups.

TABLE 2 | Executive function parameters pre and post-exercise in HTN and Non-HTN individuals (mean ± SD).

Parameter	Non-HTN		HTN		*p*-Value (effect size, η^2)		
	Pre	Post	Pre	Post	Group	Time	GxT
FLANKER							
Hits, congruent (%)†	99.9 ± 0.3	99.8 ± 0.7	99.9 ± 0.6	99.8 ± 0.6	0.93 (0.00)	0.58 (0.01)	0.94 (0.00)
Hit RT, congruent (ms)	513 ± 76	493 ± 66	510 ± 64	478 ± 63	0.63 (0.00)	**0.01 (0.35)**	0.15 (0.04)
Hits, incongruent (%)†	97.8 ± 3.0	98.0 ± 2.5	96.8 ± 4.5	95.5 ± 4.9	0.54 (0.01)	0.80 (0.00)	0.83 (0.00)
Hit RT, incongruent (ms)	606 ± 87	578 ± 82	590 ± 68	562 ± 72	0.46 (0.06)	**0.01 (0.34)**	0.89 (0.00)
Caution	0.22 ± 0.14	0.21 ± 0.12	0.24 ± 0.24	0.24 ± 0.20	0.77 (0.00)	0.97 (0.00)	0.80 (0.00)
Non-decision time (ms)	309 ± 121	296 ± 117	320 ± 83	273 ± 112	0.81 (0.00)	0.07 (0.06)	0.28 (0.02)
Attention interference (ms)	190 ± 171	152 ± 103	198 ± 176	158 ± 108	0.55 (0.01)	0.26 (0.02)	0.78 (0.00)
Perceptual strength	0.53 ± 0.19	0.55 ± 0.17	0.58 ± 0.19	0.59 ± 0.18	0.26 (0.02)	0.35 (0.02)	0.69 (0.00)
2-BACK							
Hits (%)†	67.0 ± 22.2	67.2 ± 21.5	73.5 ± 15.6	74.1 ± 17.0	0.26 (0.02)	0.96 (0.00)	0.69 (0.00)
Commission errors (%)†	6.4 ± 4.9	5.6 ± 5.2	7.3 ± 6.6	6.2 ± 5.5	0.67 (0.00)	0.07 (0.13)	0.98 (0.00)
Discriminability†	0.61 ± 0.22	0.62 ± 0.20	0.66 ± 0.15	0.679 ± 0.168	0.33 (0.02)	0.76 (0.00)	0.71 (0.00)
Hit RT (ms)	666 ± 106	651 ± 94	633 ± 89	613 ± 88	0.13 (0.04)	0.09 (0.05)	0.66 (0.00)
Caution	0.14 ± 0.02	0.15 ± 0.02	0.15 ± 0.02	0.14 ± 0.02	0.44 (0.01)	0.28 (0.02)	0.06 (0.06)
Non-decision time (ms)	402 ± 108	370 ± 95	353 ± 94	344 ± 91	0.11 (0.04)	**0.048 (0.07)**	0.27 (0.02)
Response bias	0.39 ± 0.11	0.39 ± 0.12	0.40 ± 0.11	0.39 ± 0.10	0.66 (0.00)	0.53 (0.01)	0.52 (0.01)
Drift rate-match	0.16 ± 0.03	0.17 ± 0.04	0.17 ± 0.04	0.17 ± 0.03	0.23 (0.03)	0.84 (0.00)	0.94 (0.00)
Drift rate-non-match	−0.13 ± 0.04	−0.13 ± 0.03	−0.13 ± 0.04	−0.14 ± 0.03	0.65 (0.00)	0.36 (0.02)	0.61 (0.01)
Drift rate-discriminability	0.29 ± 0.06	0.30 ± 0.04	0.31 ± 0.05	0.31 ± 0.03	0.20 (0.03)	0.43 (0.01)	0.90 (0.00)
Stimulus bias	0.03 ± 0.05	0.03 ± 0.06	0.04 ± 0.60	0.03 ± 0.04	0.70 (0.00)	0.62 (0.00)	0.56 (0.01)

Italics, Drift-diffusion modeling parameter; HTN, Hypertensive; GxT, Group-by-time interaction; RT, reaction time.
† Non-parametric effect size, Z^2/n. Bold value denotes $p < 0.05$.

DISCUSSION

Acute exercise did not alter accuracy, but increased processing speed (decreased RT), on executive function and memory tasks post-exercise in both HTN and non-HTN individuals. Our DDM data indicate that non-decision time significantly decreased post-exercise during executive function and memory tasks, and that memory stimulus bias increased post-exercise. Our data cumulatively suggest that middle-age HTN experience similar beneficial increases in executive function and memory processing speed following acute exercise as their non-HTN counterparts, and that post-exercise increases in processing speed are driven by changes outside of the decision-making process.

Hypertension and Cognitive Function

We noted no group differences in cognitive function at the $p < 0.05$ level between HTN and non-HTN in our cohort of middle-aged adults. Indeed, task accuracy and processing speeds (RT) were similar across executive function (Flanker, 2-back) and memory recognition tasks between groups. The similar executive function and memory performance between HTN and non-HTN groups contrasts with previous data (Elias et al., 1993; Li et al., 2017). These conflicting data may relate to our middle-aged sample of HTN since age independently contributes to cognitive function among hypertension (Muela et al., 2017). As such, younger HTN may not experience notable differences in cognitive function since the duration the brain is exposed to hypertension likely contributes to the degree of dysfunction (or lack thereof) (Elias et al., 1993). This suggests that our cohort of middle-aged HTN had similar cognitive health as their non-HTN counterparts, and were not currently exhibiting signs of cognitive dysfunction from accelerated brain aging. Ultimately, similar levels of brain health in adults with and without HTN may have preserved brain plasticity among HTN individuals and contributed to similar cognitive responses to acute exercise observed herein.

Our HTN cohort partially relied on anti-HTN medication to control their blood pressure to within normal levels which may attenuate differences in cognitive function between our groups (Muela et al., 2017). Whether anti-HTN therapy independently impacts cognitive function is of debate (Iadecola et al., 2016) and may depend on age, drug type, and cognitive assessment strategy (Gasecki et al., 2013; Kherada et al., 2015). The American Heart Association and American Stroke Association have recommended anti-HTN therapy for adults in mid-life and early old age as an effective means to attenuate late-life dementia (Gorelick et al., 2011). Additionally, some drug types (angiotensin II receptor blockers [ARB], angiotensin converting enzyme inhibitors [ACE-I], and calcium-channel blockers [CCB]) and combination therapies appear more effective in combating cognitive decline in HTN than other monotherapy drugs (Kherada et al., 2015). Half of our

TABLE 3 | Memory recognition parameters pre and post-exercise in HTN and Non-HTN individuals (mean ± SD).

Parameter	Non-HTN		HTN		*p*-Value (effect size, η^2)		
	Pre	Post	Pre	Post	Group	Time	GxT
MEMORY							
Hits, studied (%)†	51.8 ± 21.0	57.7 ± 20.2	53.5 ± 17.7	57.8 ± 20.7	0.71 (0.00)	**0.02 (0.12)**	0.85 (0.00)
False alarms (%)†	22.6 ± 17.7	27.2 ± 19.9	24.6 ± 17.7	30.2 ± 20.9	0.64 (0.00)	**0.01 (0.17)**	0.88 (0.00)
Discriminability	0.29 ± 0.18	0.30 ± 0.14	0.29 ± 0.16	0.28 ± 0.19	0.65 (0.00)	0.92 (0.00)	0.57 (0.01)
Hit RT (ms)	941 ± 181	897 ± 148	894 ± 149	853 ± 145	0.24 (0.03)	**0.02 (0.10)**	0.91 (0.00)
Caution	0.14 ± 0.02	0.13 ± 0.03	0.12 ± 0.02	0.13 ± 0.02	0.27 (0.02)	0.74 (0.00)	0.07 (0.05)
Non-decision time (ms)	579 ± 126	531 ± 104	564 ± 101	532 ± 136	0.79 (0.00)	**0.01 (0.14)**	0.53 (0.01)
Response bias	0.06 ± 0.02	0.07 ± 0.02	0.06 ± 0.01	0.06 ± 0.02	0.45 (0.01)	0.93 (0.00)	0.68 (0.00)
Drift rate-studied	0.01 ± 0.07	0.04 ± 0.07	0.02 ± 0.06	0.02 ± 0.11	0.95 (0.00)	0.12 (0.04)	0.26 (0.02)
Drift rate-distractor	−0.11 ± 0.08	−0.09 ± 0.07	−0.10 ± 0.09	−0.08 ± 0.10	0.75 (0.00)	0.11 (0.04)	0.87 (0.00)
Drift rate-discriminability	0.11 ± 0.08	0.12 ± 0.06	0.11 ± 0.07	0.10 ± 0.07	0.66 (0.00)	0.94 (0.00)	0.24 (0.02)
Stimulus bias	−0.10 ± 0.12	−0.05 ± 0.12	−0.08 ± 0.133	0.04 ± 0.14	0.44 (0.01)	**0.02 (0.10)**	0.76 (0.00)

Italics, Drift-diffusion modeling parameter; HTN, Hypertensive; GxT, Group-by-time interaction; RT, reaction time.
† Non-parametric effect size, Z^2/n. Bold value denotes $p < 0.05$.

hypertensive cohort were on a combination therapy (i.e., >1 anti-hypertensive drug) and 90% used either an ARB, ACE-I, or CCB, which may have contributed to the similar cognitive health between our HTN and non-HTN groups. Taken together, these data indicate that our sample of medicated, well-controlled, middle-aged HTN had similar cognitive function as their non-HTN counterparts, potentially contributing to similar cognitive responses to acute exercise.

Hypertension, Exercise, and Cognitive Function

To our knowledge, this is the first study to investigate the acute effects of exercise on cognitive function in adults with hypertension, a population at-risk for cognitive decline. Accuracy on executive function tasks (Flanker and 2-back) was unaltered by acute exercise in both HTN and non-HTN groups at the $p < 0.05$ level. We did note, however, a significant increase in memory recognition hits and false alarms post-exercise in both HTN and Non-HTN individuals. Although the percentage of correctly identified "studied" words (i.e., hits) increased post-exercise, this is not indicative of improved memory recognition performance or accuracy *per se* since it was accompanied by an increase in the percentage of false alarms (incorrect responses where distractor words were classified as "studied"). This seems to suggest that acute exercise altered how individuals categorized memory stimuli and will be discussed further below with insight from DDM.

The small effects of acute exercise on cognitive task accuracy observed herein concurs with previous experimental data (Tsai et al., 2014; Ji et al., 2017; Tsukamoto et al., 2017). Meta-analytical investigations suggest there may be a small effect on non-time dependent cognitive task performance (i.e., accuracy) (Ludyga et al., 2016), although this is not universal (McMorris and Hale, 2012). Improvements in cognitive task accuracy post-exercise may be difficult to observe as many studies rely on tasks vulnerable to ceiling effects (i.e., task difficulty is not sufficient that improvements in cognitive processing or decision-making can alter hit rates). Indeed, this may impact our findings with the Flanker task (hit rate ≈ 99%), although ceiling effects likely did not impact the effect of exercise on 2-back or memory recognition tasks based on the lower mean hit rates (≈70 and 53%, respectively). These data indicate that acute aerobic exercise does not statistically improve accuracy on executive function or memory tasks in middle-aged adults with and without hypertension.

We noted faster executive function and memory processing speed post-exercise in both adults with and without hypertension. Accelerated processing speed post-exercise manifested as significantly reduced RT for Flanker/memory recognition tasks, with smaller effects (statistical trends) for 2-back. This facilitation of RT is in-line with recent literature (Tsai et al., 2014; Ji et al., 2017; Tsukamoto et al., 2017) as well as meta-analyses of the literature at-large that indicate RT is sensitive to changes with acute exercise (Tomporowski, 2003; Ludyga et al., 2016). Previous findings suggest larger benefits of exercise on RT are seen in children and older adults, with attenuated benefits in young healthy adults (Ludyga et al., 2016). While the effect of acute exercise on cognitive function is under-investigated among middle-aged adults (Ludyga et al., 2016), our observation of accelerated RT post-exercise suggests middle-aged adults exhibit similar facilitation of post-exercise RT as children and older adults. These data suggest that middle-aged, medicated HTN experience similar facilitation of cognitive processing speed (reduced RT), on executive function, and memory tasks as their non-HTN counterparts following acute exercise.

Contrary to our hypothesis, exercise elicited similar beneficial effects on cognitive function (faster cognitive processing speeds) in HTN and Non-HTN adults. Similar cognitive responses to acute exercise in HTN and Non-HTN adults may relate to similar vascular health. Vascular health has been directly linked to

both brain health and cognitive function (Gorelick et al., 2011). Our HTN cohort utilized both medication (anti-HTN, statins) and physical activity to achieve adequate blood pressure control (Lefferts et al., 2018a,b). This combined approach may have had favorable effects on their vascular health (Gepner et al., 2017). Indeed, our sample of HTN had similar vascular stiffness and cerebrovascular responses to cognitive activity as their Non-HTN counterparts (Lefferts et al., 2018a). Moreover, acute exercise had similar effects on the cerebrovasculature in our HTN and Non-HTN adults (Lefferts et al., 2018b). As such, brain health and plasticity may have been partially supported in this sample of HTN through vascular health, ultimately giving way to similar cognitive responses to exercise.

Exercise, Cognitive Function, and Insight From Drift-Diffusion Modeling

Previous studies have provided limited insight into psychological factors underlying exercise-induced changes in cognitive function. A reason for this may be due to reliance on standard metrics of cognitive performance (task accuracy and RT) (Ludyga et al., 2016). Any change in cognitive function could stem from multiple components of the decision-making process that ultimately influence RT. Mathematical modeling via DDM is a novel means of dissecting the entire decision-making process contained in the behavioral data into its underlying components of visual encoding, evidence accumulation and decision, and motor response. As such, DDM may offer insight into mechanisms behind changes in cognitive performance (hits and false alarms) and mechanisms of accelerated RT following exercise by quantifying the changes in the decision-making process that elicited the observed responses.

We noted no statistically significant effect of acute exercise on executive function task accuracy, as assessed by hit rate on the Flanker and 2-back. The ability to correctly identify stimuli in a given task is strongly dependent on the strength of evidence extracted from the stimuli itself (White et al., 2016). An increase in the strength of evidence extracted from the stimuli would be expected to increase the hit rate and potentially decrease RT (stronger evidence reaches decision boundary faster). Since Flanker and 2-back hit rates were statistically unaltered by exercise, it is not surprising that the strength of evidence (drift rate) did not change post-exercise for either executive function task.

We did, however, observe increases in hit and false alarm rates during the memory recognition task post-exercise. This effect of exercise on task performance reflects a change in how evidence was extracted from the stimuli (quantified as drift rate by DDM). Indeed, DDM revealed that drift rates for both old/studied and new/distractor words increased (non-significantly) post-exercise. According to the model, greater absolute drift rates indicate stronger perceived evidence for a given stimuli (positive drift rates for "old/studied," and negative for "new/distractor" words). The drift rates of old/studied and new/distractor words were summed to create an index of stimulus evaluation bias, which significantly increased post-exercise. A change in stimulus evaluation bias signifies a shift in the memory criterion, altering how the stimulus is processed and what evidence is extracted from the stimuli under consideration (White and Poldrack, 2014). An increase in stimulus evaluation bias (and thus, a more positive value) suggests all items seemed to provide more evidence as old/studied even if they were new/distractor stimuli. A more liberal memory criterion (i.e., require weaker memory/less evidence to identify word as "old/studied") may be preferred when memory items are perceived as being harder to remember (Starns et al., 2012). Thus, individuals may have perceived the memory task as more difficult post-exercise and adapted a more lenient memory criterion, thereby inducing a stimulus evaluation bias and resulting in a tendency to identify all items as "old/studied" (increasing hits/false alarms). Whether the post-exercise changes in stimulus evaluation bias are a direct result of exercise or the experimental design itself is unclear and requires further scrutiny.

Significant reductions in post-exercise executive function (Flanker, trend for 2-back) and memory RT could stem from changes in encoding, the decision process itself, and motor execution. DDM was able to provide insight into the components of RT that are altered by acute exercise. We noted significant reductions in non-decision time during the 2-back and memory recognition tasks, with smaller effects trending toward statistical reductions during the Flanker task. As such, reductions in executive function and memory recognition RT observed herein were likely related to significant reductions in non-decision time (the sum of encoding and motor response phases that occur immediately prior to, and directly following, the actual decision making process). Previously, it was unclear if changes in RT post-exercise stemmed from alterations in stimulus evaluation and response selection (decision), or motor execution (Tsai et al., 2014). Neuroelectric proxies of stimulus evaluation duration (P3 latency; likely analogous to the decision component of DDM), may not be altered by exercise (Chu et al., 2015) although this is not universal (Kamijo et al., 2009). Our data indicate that improved executive function and memory processing speed post-exercise is largely independent from changes in the decision-making process itself (evidence strength, caution, bias) and is isolated to the encoding and motor response.

While we are unable to directly comment on whether exercise increases cognitive processing speed via accelerated visual encoding, motor response, or a combination of both, there is evidence that each may be altered post-exercise. Limited data suggest there are small changes in the time required for a motor response post-exercise (Ando et al., 2009) which could contribute to shorter non-decision time. It is unclear if accelerated encoding may explain the remaining reductions in post-exercise non-decision time. While, time required for visual encoding may decrease post-exercise owing to the residual effects of exercise on sensory cortex excitability (Bullock et al., 2017), some data suggest exercise does not alter neuroelectric indices of the initial sensory extraction from a cognitive stimulus (Chu et al., 2015). Additional contributors to post-exercise facilitation of non-decision time may include acute elevation of brain-derived neurotrophic factor and acute catecholamine/endorphin-mediated increases in arousal (McMorris et al., 2008; Dishman and O'Connor, 2009).

Ultimately, the mechanisms behind the contributions of visual encoding and motor response to post-exercise changes in non-decision time, and in turn to RT, require further research.

Implications

Our data suggest that brain plasticity, the characteristic that allows the brain to respond to a stimulus and improve processing speed (in this case, a single bout of exercise), is preserved in well-controlled, middle-aged HTN. Studying the neurocognitive response to a single bout of exercise is often viewed as a first step toward understanding how to favorably modulate resting functional state with habitual exercise training (Basso and Suzuki, 2017). In the present study, well-controlled non-obese HTN experienced similar beneficial increases in cognitive function following acute exercise as their non-HTN counterparts. A previous study from Teixeira et al. has noted no beneficial effects of a 12-week exercise program on cognitive function in resistant overweight/obese HTN (Teixeira et al., 2015). Additional research is needed to explore the relationship between acute exercise-cognitive responses and exercise training-cognitive adaptations in order to understand how to best use exercise as an adjuvant for improving cognitive health in HTN.

Prolonged sitting has been shown to have a detrimental effect on the brain, acutely reducing cognitive performance (Baker et al., 2018; Carter et al., 2018; Garcia-Hermoso et al., 2018). Time spent is sedentary pursuits may be slightly more detrimental to executive function in HTN adults compared to non-HTN adults (Steinberg et al., 2015). Replacing time spent in sedentary pursuits with physical activity/exercise has been shown to prevent acute declines in cognitive performance (Baker et al., 2018; Carter et al., 2018; Garcia-Hermoso et al., 2018) which may have implications for workplace productivity in non-HTN adults (von Thiele Schwarz and Hasson, 2011). Our findings may extend to HTN adults and suggest that if well-controlled, hypertension will not prevent the HTN adult from realizing the acute beneficial effects of exercise on cognitive function.

Limitations

Our HTN group did not refrain from taking their anti-HTN medication during our study which may have influenced our findings. It is currently unclear if *acute* ingestion of anti-HTN medication directly impacts cognitive function. While acute doses of an ARB have been reported to enhance prospective memory in young normotensive adults (Mechaeil et al., 2011), this remains to be replicated in a clinically relevant population (i.e., hypertension). As such, future research should investigate the effects of acute anti-HTN medication on cognitive function following acute exercise. Whether responses are different among a group of older, or un-medicated HTN is additionally unclear and requires further research. We purposefully chose to interrogate the effect of exercise on cognitive function from 10 to 30 min post-exercise since we wished to identify if HTN individuals experienced the same benefits of exercise on cognitive function as their non-HTN counterparts and meta-analyses indicate this time period is sensitive to the acute effects of exercise (Chang et al., 2012). It is possible that further or differential changes in cognitive function could occur in these groups during prolonged recovery (i.e., >30 min) from a single bout of exercise. While this study was sufficiently powered to detect the larger effects of exercise on processing speed (RT), it may not have been adequate to detect subtle effects of both hypertension and exercise on processes of decision-making that might be identified with a larger sample. As such, larger studies utilizing similar novel methods may be required to reveal the potentially more subtle effects of hypertension and acute exercise on latent aspects of decision-making.

CONCLUSIONS

We sought to investigate the effects of exercise on cognitive function in middle-aged HTN and non-HTN individuals and examine the effect of exercise on underlying processes of decision-making (via DDM). Both HTN and non-HTN individuals had similar cognitive responses following a single exercise bout of a recommended dose/intensity. While acute exercise did not alter task accuracy significantly, there were significant reductions in post-exercise RT for both executive function and memory domains in both groups. DDM revealed that changes in RT may largely stem from significant reductions in non-decision time which encompasses the early (visual encoding) and late (motor response) portions of the decision-making process.

AUTHOR CONTRIBUTIONS

WL, CW, and KH conceived and designed research. WL and JD collected and analyzed the data. All authors interpreted results. WL prepared figures and drafted manuscript. All authors edited, revised, and approved the final version of the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnagi.2019.00041/full#supplementary-material

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Skeletal Muscle Fiber Size and Gene Expression in the Oldest-Old With Differing Degrees of Mobility

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The oldest-old, in the ninth and tenth decades of their life, represent a population characterized by neuromuscular impairment, which often implies a loss of mobility and independence. As recently documented by us and others, muscle atrophy and weakness are accompanied by an unexpected preservation of the size and contractile function of skeletal muscle fibers. This suggests that, while most fibers are likely lost with their respective motoneurons, the surviving fibers are well preserved. Here, we investigated the mechanisms behind this fiber preservation and the relevance of physical activity, by comparing a group of 6 young healthy controls (YG: 22–28 years) with two groups of oldest-old (81–96 years), one able to walk (OW: n = 6, average 86 years) and one confined to a wheelchair (ONW n = 9, average 88 years). We confirmed previous results of fiber preservation and, additionally, observed a shift in fiber type, toward slow predominance in OW and fast predominance in ONW. Myonuclear density was increased in muscles of ONW, compared to YG and OW, potentially indicative of an ongoing atrophy process. We analyzed, by RT-qPCR, the expression of genes relevant for fiber size and type regulation in a biopsy sample from the vastus lateralis. In all oldest-old both myostatin and IGF-1 expression were attenuated compared to YG, however, in ONW two specific IGF-1 isoforms, IGF-1EA and MGF, demonstrated a further significant decrease compared to OW. Surprisingly, atrogenes (MURF1 and atrogin) expression was also significantly reduced compared to YG and this was accompanied by a close to statistically significantly attenuated marker of autophagy, LC3. Among the determinants of the metabolic fiber type, PGC1α was significantly reduced in both OW and ONW compared to YG, while AMPK was down-regulated only in ONW. We conclude that, in contrast to the shift of the balance in favor of pro-atrophy factors found by other studies

in older adults (decreased IGF-1, increase of myostatin, increase of atrogenes), in the oldest-old the pro-atrophy factors also appear to be down-regulated, allowing a partial recovery of the proteostasis balance. Furthermore, the impact of muscle activity, as a consequence of lost or preserved walking ability, is limited.

Keywords: aging, oldest-old, physical activity, muscle atrophy, single muscle fibers, myonuclei, gene expression

INTRODUCTION

Aging is accompanied by a decline in skeletal muscle mass and force with a significant impact on the everyday quality of life of the elderly (Janssen et al., 2002; Metter et al., 2002; Narici and Maffulli, 2010; Sayer et al., 2013). With advancing age, the architecture of skeletal muscle tissue is altered, with an increase of intramuscular adipose tissue, changes in the angle of pennation (Narici et al., 2003) and, moving down to cellular level, is associated with a reduction in muscle fiber cross sectional area, which becomes more pronounced when combined with disuse and inactivity (D'Antona et al., 2003; Suetta et al., 2007, 2009; Hvid et al., 2011). Of note, most of the available information on muscle aging comes from studies on individuals in the age range of 65–75, often identified as older-adult or young-old (Frontera et al., 1991, 2000; Larsson et al., 1997; Hughes et al., 2001; D'Antona et al., 2003; Ochala et al., 2007; Yu et al., 2007). However, in a recent study (Venturelli et al., 2015), members of our group examined not only much older subjects (∼90 years), usually identified as oldest-old, but also compared subjects characterized by different levels of mobility. The ninth decade of life is characterized by an accelerated decline of many functions and the loss of independent mobility is a common condition in non-agenarians. Indeed, according to epidemiological surveys, only approximately 30% of this segment of population is still able to walk (Christensen et al., 2008; Berlau et al., 2009). Our study design facilitated the isolation of the impact of disuse and advanced aging on muscle structure and function as we compared oldest-old subjects confined to a wheelchair with subjects still able to walk. Surprisingly, we found that the single fiber cross sectional area was preserved in both the oldest-old groups, while, in contrast, whole muscle volume and maximal voluntary force were dramatically reduced compared to young healthy subjects, particularly in the lower limbs of those who had lost the ability to walk. This observation was in partial agreement with the findings of Trappe and coworkers (Raue et al., 2009; Grosicki et al., 2016) who also studied a population of oldest-old individuals (∼85 years) and observed a preservation of skeletal muscle fiber size and a maintained or even improved capacity of single fibers to develop force during maximal calcium activation *in vitro*.

The conundrum of greatly diminished muscle size and function, while individual muscle fiber size and function are preserved, may potentially be explained by a loss of muscle fibers. In this respect, the neural system plays a pivotal role. Initially, with progressive motoneuron death and fiber denervation, and, then, by the disappearance of the denervated fibers or, possibly, by partial reinnervation of the surviving fibers by sprouting of slow motoneurons (Delbono, 2003, 2011; Payne and Delbono, 2004; Aagaard et al., 2010; Reid et al., 2012; Venturelli et al., 2018). Interestingly, it is still debated whether the loss of motoneurons can be slowed down by regular physical activity [see Power et al. (2010) in favor and Piasecki et al. (2016) against this view]. Unfortunately, the direct assessment of the impact of neural events on muscle fiber size and number during advanced age and disuse is somewhat complicated (Doherty et al., 1993). However, the comparison between the force developed during maximal voluntary contraction (MVC) and electrically stimulated contraction helps to estimate the contribution of reduced neural drive to muscle deconditioning (Venturelli et al., 2015). Furthermore, the evaluation of *in vivo* single twitch kinetics may further contribute to understand the functional condition of skeletal muscle, as the maximal rates of force development are clearly different among slow and fast motor units (Mero et al., 1991). Unfortunately, information regarding single twitch kinetics in the oldest-old is sparse.

Skeletal muscle fiber size is the result of a balance between protein synthesis and degradation and these two processes are regulated by very specific signaling pathways. Indeed, according to recent reviews of the literature in this field (Blaauw et al., 2013; Ciciliot et al., 2013), two major signaling pathways control protein synthesis, the IGF1–AKT– mTOR pathway, acting as a positive regulator, and the myostatin–Smad2/3 pathway, acting as a negative regulator. In turn, two major processes are responsible of protein degradation, the proteasomal and the autophagic–lysosomal pathways. The latter processes are controlled by a number of factors including FoxO transcriptional factors, atrogenes, and NF-kB (Schiaffino et al., 2013). However, the balance between these signaling pathways for the homeostatic control of skeletal muscle fiber size during advanced aging is not fully understood.

Therefore, this study sought to provide insight into the conundrum of whole muscle dysfunction and atrophy, but preservation of single fiber size by elucidating the expression of genes responsible for regulating skeletal muscle size. The analysis was carried out in a group of oldest-old people still able to walk and in a group confined to a wheelchair for at least 2 years, to assess the relevance of a preserved motor activity. We tested the hypothesis that, independent of the progressive neural system impairment associated with both advanced age and disuse, the balance between signals supporting protein synthesis and stimulating protein degradation is partially maintained. This balance results in the preservation of fiber size in the surviving muscle fibers, despite the marked overall loss of muscle fibers, the large reduction in whole muscle mass, and the decline in muscle force.

MATERIALS AND METHODS

Participants

Eight young subjects (YG), 22–28 years old, and 15 oldest-old people, 81–96 years old, were enrolled in this study. The general characteristics of subjects are reported in **Table 1**. The oldest-old participants were approved to partake in this study based upon a physician's assessment of minimal cognitive, cardiovascular, and musculoskeletal disease. This screening included, a health history, a physical examination, an evaluation of balance during sitting and ambulation (Tinetti, 1986), a blood pressure assessment, blood analyses, and a familiarization with the study procedures. The Ethics Committee of the Department of Neuroscience, Biomedicine and Motor Science of the University of Verona approved the study, and all experimental procedures were performed in accordance with the Declaration of Helsinki. Written, informed consent was obtained from all participants before inclusion in the study. The young subjects were normally physically active college students. The oldest-old participants were classified according to their ambulation capacity (Tinetti, 1986). Specifically, community dwelling subjects, able to walk independently (81–96 years old; n = 6) are identified here as OW, while, the oldest-old subjects with severe mobility limitation (81–92 years old; n = 9) are identified as ONW. The subjects were recruited among residents of the Monsignor Arrigo Mazzali Foundation, Geriatric Institute of Mantua, Italy. The medical staff recruited the oldest-old subjects that, at the time of the study, were mobility limited in the lower limbs (at least 2 years of being wheelchair bound) and unable to stand-up from a chair and walk independently (Tinetti, 1986). The diagnosis of altered gait capacity, poor balance, and an elevated risk of falling were the cause of this mobility restriction. Thus, three groups were formed and comparisons were carried out for each parameter between the three groups (see statistical analysis).

TABLE 1 | Subject characteristics.

	YG = 8	OW = 6	ONW = 9
Age (years)	24 ± 3	86 ± 4 *	88 ± 5 *
Sex (F/M)	5/3	4/2	6/3
POMA Balance test (0–16)	16 ± 0	16 ± 0	3 ± 1
POMA Gait test (0–12)	12 ± 0	12 ± 0	0 ± 0
Body mass (kg)	61 ± 13	57 ± 12	57 ± 12
Body height (m)	1.66 ± 0.08	1.63 ± 0.07	1.65 ± 0.09
BMI (kg m^{-2})	22 ± 3	21 ± 4	21 ± 3
Body fat (%)	23 ± 3	32 ± 4 *	39 ± 5 *†
Sarcopenia index (kg m^{-2})	7.5 ± 2.3	5.4 ± 1.7	5.1 ± 0.6
Thigh muscle mass (kg)	5.3 ± 0.3	4.3 ± 0.3 *	3.3 ± 0.4 *†
Glucose (mg dl^{-1})	85 ± 4	96 ± 7 *	95 ± 6 *
RBC (10^6 μl^{-1})	4.2 ± 0.1	4.1 ± 0.3	4.0 ± 0.3
Hb (g dl^{-1})	13.5 ± 0.5	11.5 ± 0.4 *	11.3 ± 0.7 *
HDL (mg dl^{-1})	50 ± 9	51 ± 8	53 ± 7
LDL (mg dl^{-1})	95 ± 11	106 ± 10 *	111 ± 13 *
SBP (mmHg)	119 ± 5	134 ± 6 *	137 ± 5 *
DBP (mmHg)	81 ± 5	84 ± 5	88 ± 9
Cognitive function MMSE (0–30)	–	27 ± 3	25 ± 3
Comorbidity N (%)			
Cardiovascular diseases	–	2 (33)	1 (11)
Diabetes	–	2 (33)	2 (22)
COPD	–	1 (17)	1 (11)
Pharmacological treatments N (%)			
Anti-depressive agents (Trazodone)	–	2 (33)	3 (33)
Oral anti-diabetics (Thiazolidinedione)	–	1 (17)	2 (22)

*YG, Young; OW, walking oldest-old, ONW, non-walking oldest-old; F, female; M, male; POMA, Performance-oriented assessment of mobility problems in elderly patients (Tinetti, 1986); BMI, body mass index, Sarcopenia index (Newman et al., 2003); RBC, red blood cells; Hb, hemoglobin; HDL, high-density lipoprotein; LDL, low-density lipoprotein; SBP, systolic blood pressure; DBP, diastolic blood pressure; MMSE, mini-mental state examination (Folstein et al., 1975); COPD chronic obstruction pulmonary disease. *Significantly different from YG; †Significantly different from OW.*

Cognitive Function

The *Mini Mental State Examination (MMSE)* was used to assess the global cognitive function of the participants (Folstein et al., 1975).

Body Composition

Body fat and lean mass were assessed by means of DXA using a total body scanner (QDR Explorer W, Hologic, MA, United States; fan-bean technology, software for Windows XP version 12.6.1) according to the manufacturer's procedures. The scanner was calibrated daily against the standard supplied by the manufacturer to avoid possible baseline drift. Whole body scanning took approximately 7 min. Data were analyzed using standard body region markers: upper and lower extremities, head, and trunk (pelvic triangle plus chest or abdomen). Additionally, the DXA scans were examined using non-standard body region markers to define thigh segments. The thigh region was delineated by an upper border formed by an oblique line passing through the femoral neck to the horizontal line passing through the knee (Skalsky et al., 2009). In agreement with the European consensus on definition and diagnosis of sarcopenia (Cruz-Jentoft et al., 2018), the level of sarcopenia was calculated as appendicular lean mass (aLM) corresponded to the sum of lean mass in the arms and legs, relative to height squared (Newman et al., 2003). All scanning and analyses were performed by the same operator to ensure consistency. In our laboratory the precision error (percent coefficient of variation with repositioning) of whole-body DXA measurements is 2.3, 0.5, and 2.8% for fat mass, lean mass, and percent fat mass, respectively.

Electromyography

M-waves were recorded during femoral nerve stimulation in the vastus lateralis and biceps femoris muscles (detailed in next section). Pairs of full-surface solid adhesive hydrogel electrodes (H59P, Tyco Healthcare Group, Mansfield, MA, United States) were positioned lengthwise over the muscle belly, with an inter-electrode distance (center-to-center) of 20 mm. The ground electrodes were fixed over the ipsilateral patella. Light skin abrasion followed by skin cleansing kept electrical impedance below 10 kΩ. EMG signals were amplified with a pass-band of 10 Hz–1 kHz and digitized online at a sampling frequency of 5 kHz.

Nerve Stimulation, Twitch Force and Kinetics

To record electrically evoked contractile responses of the quadriceps muscle, subjects were seated in an upright position with back support, in a custom made ergometer, as previously described (Venturelli et al., 2015). The hip and the knee were flexed at 90°, with the right ankle attached, via a strap and rigid steel bar, to a force transducer (DBBSE-100 kg, A2829. Applied Measurements Limited, Aldermaston, Berkshire, United Kingdom). The output from the force transducer was amplified (INT2-L, London Electronics Limited, Sandy Bedfordshire, United Kingdom), and recorded at a sampling rate of 5 kHz with a PowerLab-16/35 data acquisition system (ADInstruments, Bella Vista, NSW, Australia). Each test procedure began with the determination of the maximal M-wave and force responses. Briefly, current intensity was progressively increased from 0 mA to the value beyond which there was no further increment in M-wave amplitude. The stimulus utilized for the study was set at the 125% of the intensity required to produce a maximal M-wave response. To record the twitch response of the quadriceps muscle, the femoral nerve was stimulated with the cathode positioned in the femoral triangle, 3–5 cm below the inguinal ligament, and the anode placed over the iliac crest. The resting twitches (RT) were evoked in the passive muscle using electrical stimulation consisting of single square-wave pulses of 0.1 ms duration, delivered by a Digitimer DS7 constant-current stimulator (Digitimer Ltd., Welwyn Garden City, United Kingdom). Twitch peak amplitude, time to peak, maximal rate of force development between 10 and 90% of twitch peak (MRFD), maximal relaxation rate between 90 and 10% of twitch peak (MRFR), MFRD and MRFR relative to twitch peak (MRFD/F; MRFR/F), were determined for all RT (Sandiford et al., 2005; Maffiuletti et al., 2016).

Skeletal Muscle Sampling

Skeletal muscle samples were obtained with a tru-cut needle (Paoli et al., 2010) from the vastus lateralis. Muscle samples were divided in three parts, the first immediately frozen in liquid nitrogen and used for molecular analysis, the second embedded in O.C.T. compound (Tissue-Tek), frozen in liquid nitrogen-cooled isopentane and used for morphological analysis, and the third immersed in skinning solution with 50% glycerol for single fiber dissection and myonuclei analysis. The samples were then stored at −80°C or at −20°C, if immersed in skinning solution (see below), with glycerol.

Morphometry

Muscle samples, embedded in O.C.T., were cut into 10 μm thick cryosections with a cryostat (Thermo Fisher Scientific™ CryoStar™) maintained at −20°C and mounted on glass slides. Muscle sections were air-dried at room temperature rinsed 3 times for 5 min in 10% phosphate-buffered saline (PBS) and incubated for 60 min in goat serum at room temperature. Sections were then incubated for 1 h at room temperature with the primary antibody BA-F8 [1:50] against MHC type I. Following incubation with the primary antibody, the sections were rinsed 3 times for 5 min with PBS buffer and incubated for 1 h at room temperature with secondary antibody A21140 Alexa Fluor 350 goat anti mouse [1:800]. Then, the sections were rinsed 3 times for 5 min with PBS. Subsequently, the sections were incubated for 1 h at room temperature with primary antibodies SC-71 [1:200] against MHC type II, and ab11575 [1:200] against laminin. Following incubation with the primary antibodies, the sections were rinsed 3 times for 5 min with PBS buffer and incubated for 1 h at room temperature with secondary antibodies A21121 Alexa Fluor 488 goat anti mouse [1:800] and Ab96884 DyLight 550 goat anti rabbit [1:800]. Successively, the sections were rinsed 3 times for 5 min with PBS buffer and then dH_2O.

BA-F8 and SC-71 antibodies were purchased from Development Studies Hybridoma Bank. Anti-laminin antibodies were purchased from the Abcam company. Secondary antibodies were purchased from Abcam and Invitrogen. Slides were visualized with an Axio Observer Z1 microscope (Carl Zeiss) using conventional wide field fluorescence microscopy as well as optical sectioning via structured-illumination fluorescence microscopy (Apotome, Carl Zeiss). The microscope was equipped with green (Excitation: BP 470/40 nm; Emission BP 525/50 nm) and blue (Excitation: BP 365/12 nm; Emission LP 397 nm) filters, an AxioCam HRm camera, and AxioVision software (Carl Zeiss). For fiber type distribution, all muscle fibers, on the cross section, were analyzed and a mean of 277 fibers were counted for each subject. Selected areas, corresponding to a mean of 91 fibers, were photographed and used for determination of fiber area using the public domain image–processing software, Image-J v1.46r (National Institute of Health, Bethesda, MD, United States).

Myonuclei Counting

Single muscle fibers were manually dissected from the fiber bundles, immersed in skinning solution (mM composition: K propionate 150, Mg acetate 5, Na ATP 5, EGTA 5 and KH2PO4 5; relaxing solution KCl 100, imidazole 20, MgCl2 5, Na ATP 5 and EGTA 5), and fixed with 4% paraformaldehyde in PBS for 20 min at room temperature. After a short permeabilization with 0.1% Triton X-100 in PBS at room temperature, the fibers were incubated in 10% normal goat serum for at least 30 min to block non-specific antibody binding. Staining of Z lines with an anti α-actinin antibody was carried out to visualize the fiber segment and measure its size. Mouse anti α-actinin (clone EA-53 Sigma) was applied (1:2000) at room temperature in PBS, followed after

3 washes (10 min each), by fluorescent secondary Alexa-568 anti-mouse, (Molecular Probes) for 2 h at room temperature. To visualize nuclei, single fibers were stained with Hoechst (25 μg/ml; SIGMA) for 10 min. After a final wash in 0.1 M PB, the fibers were mounted in 100% glycerol (Sigma-Aldrich) and covered with a coverslip. The fibers were viewed with a confocal microscope (VICO, Nikon). Serial confocal optical sections (step size: 0.5 μm) were collected by scanning the fiber on the *z* axis from top to bottom. The fiber segment volume was reconstructed by adding the volume of the individual sections, each obtained as the product of thickness section (*z* axis) by surface area (*xy* axis). The sections were then collapsed on the *z* axis and the number of nuclei was counted. From nuclei number and fiber segment volume, the nuclear density (nuclei/10^6 μm^3) and the nuclear domain size (μm^3/nucleus) were obtained. From fiber segment length and nuclei number, the longitudinal density (nuclei/mm) of the nuclei was determined.

RNA Preparation and Analysis

High-quality RNA from muscle tissues was isolated using the RiboPure RNA Purification Kit (Ambion, Life Technologies, United States) according to the manufacturer's instructions. The cDNA was prepared using the High Capacity cDNA Reverse Transcription kit (Applied Biosystems, Life Technologies, United States); the quantitative real-time PCR (RT-qPCR) was performed using the SYBR Green PCR Master mix (Applied Biosystems, Life Technologies, United States) according to the protocol for use in the Applied Biosystems 7500 Real-Time PCR System. For the quantification analysis, the comparative threshold cycle (Ct) method was used. The Ct values of each gene were normalized to the Ct value of cyclophilin in the same RNA sample. The gene expression levels were evaluated by fold change using the equation 2^{-ddCt}. The primers used are reported in **Table 2**.

TABLE 2 | Primers used in RNA analysis.

AKT1	Forward: 5′-CTGGTGCATCAGAGGCTGT-3′ Reverse: 5′-TTGATGTACTCCCCTCGTTTG-3′
AKT2	Forward: 5′-ACACAAGGAAAGGGAACCAG-3′ Reverse: 5′-ACCTAGCTCGGGACAGCTC-3′
AMPKα2	Forward: 5′-TACATTCTGGGTGACACGCT-3′ Reverse: 5′-TCCTACCACATCAAGGCTCC-3′
Atrogin	Forward: 5′-GCAGAGGCTGAGCGACGGG-3′ Reverse: 5′-GTTTGCGGATCTGCCGCTCG-3′
CyclophilinA or PPIA	Forward: 5′-TGTTCTTCGACATTGCCGT-3′ Reverse: 5′-TCTGTGAAAGCAGGAACCCT-3′
GDF-11	Forward: 5′-CTGGAGGAGGACGAGTACCA-3′ Reverse: 5′-GAACATCACCTTGGGGCTGA-3′
FOXO3A	Forward: 5′-CTACGAGTGGATGGTGCGTT-3′ Reverse: 5′-TCTTGCCAGTTCCCTCATTC-3′
IGF-1	Forward: 5′-GCTCTTCAGTTCGTGTGTGG-3′ Reverse: 5′-CGCAATACATCTCCAGCCTC-3′
IGF-1EA	Forward: 5′-GACATGCCCAAGACCCAGAAGGA-3′ Reverse: 5′-CGGTGGCATGTCACTCTTCACTC-3′
LC3 or MAP1LC3A	Forward: 5′-GCGACCAGCACCCCAGCAAA-3′ Reverse: 5′-GCGGCGCCGGATGATCTTGA-3′
IGF-1EC/MGF	Forward: 5′-CGAAGTCTCAGAGAAGGAAAGG-3′ Reverse: 5′-ACAGGTAACTCGTGCAGAGC-3′
mTOR	Forward: 5′-CATTGTTCTGCTGGGTGAGA-3′ Reverse: 5′-TCCGGCTGCTGTAGCTTATT-3′
MURF-1 or TRIM63	Forward: 5′-ACGAGGTGATCATGGATCGT-3′ Reverse: 5′-CTTCGTGCTCCTTGCACAT-3′
Myostatin or GDF-8	Forward: 5′-TGTAACCTTCCCAGGACCAG-3′ Reverse: 5′-AGAGGGTAACGACAGCATCG-3′
PGC-1α	Forward: 5′-GGTGCAGTGACCAATCAGAA-3′ Reverse: 5′-AATCCGTCTTCATCCACAGG-3′
Sirt-1	Forward: 5′-GCTCGCCTTGCTGTAGACTT-3′ Reverse: 5′-TGTGACAGAGAGATGGCTGG-3′

Statistical Analysis

The data collected in each group of subjects are presented as the mean ± standard deviation (s.d.) or standard error of the mean (s.e.m.), as indicated. Statistical significance of the difference between means was determined with repeated measures ANOVA followed by Bonferroni *post hoc* test. GraphPad Prism software was used for all analysis.

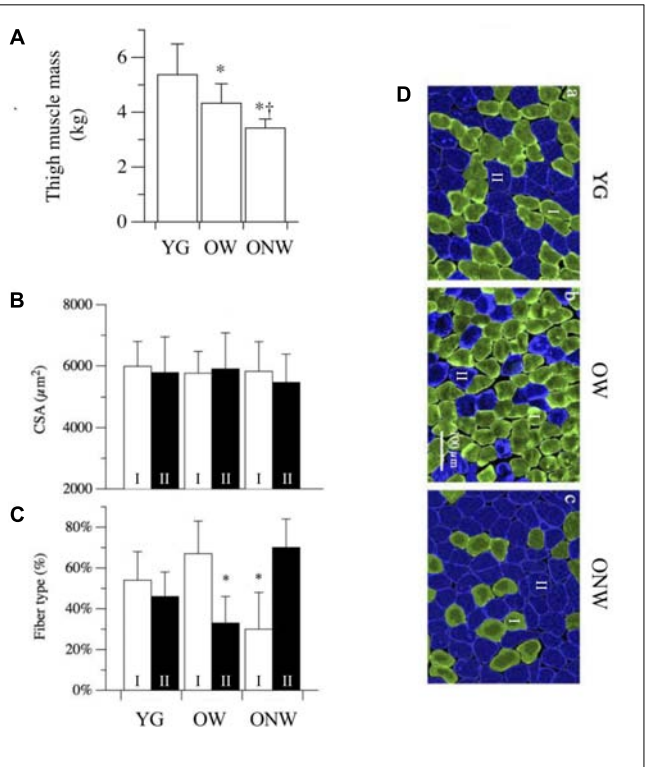

FIGURE 1 | Variations in thigh muscle mass, fiber cross sectional area and fiber type distribution in vastus lateralis of young (YG), walking oldest-old (OW), and non-walking oldest-old (ONW). **(A)** Thigh muscle mass, determined with DEXA, documents the significantly lower values in OM and OI compared to YG and even lower values in ONW compared to OW (YG $n = 8$, OW $n = 6$, and ONW $n = 9$). **(B)** Single muscle fiber cross sectional area was not different between the three groups. **(C)** Muscle fiber types, determined with anti-myosin antibodies, documents a significant difference in OW and ONW compared to YG. **(D)** Examples of sections of biopsy samples stained with anti-slow myosin antibody (green) and anti-fast myosin antibody (blue). Data in panels **A–C** are presented as mean ± S.E.; *Significantly different from YG subjects; †Significantly different from OW subjects.

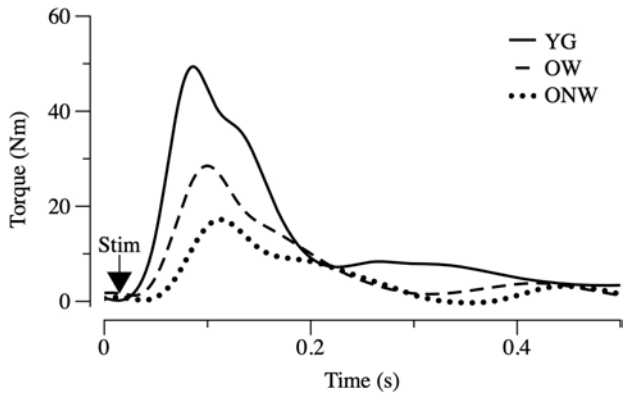

FIGURE 2 | Electrically evoked twitch in young (YG), walking oldest-old (OW), and non-walking oldest-old (ONW). Examples of twitch recorded in leg extensor muscles after single pulse stimulation are illustrated. Protocol of stimulation and torque recording are described in Section "Materials and Methods." Values of the time and torque parameters are reported for the three groups in **Table 3**.

RESULTS

Muscle and Fiber Atrophy

To characterize the degree of muscle atrophy we determined thigh muscle mass and muscle fiber cross sectional area in the three groups of subjects (YG, OW, ONW). Overall both OW and ONW participants were classified as sarcopenic according to the cut-points of 7.23 kg m^{-2} (men) and 5.67 kg m^{-2} (women) (**Table 1**). As documented in **Figure 1A**, the thigh muscle mass, determined with DXA, revealed significantly lower values in the old compared to young ($p < 0.05$) and even lower values in ONW compared to OW ($p < 0.05$). In contrast, the determination of single muscle fibers cross sectional area did not reveal significant differences among the average values in the three groups (**Figure 1B**). The counting of the fibers, identified as slow or fast with specific anti-myosin antibodies, revealed that, in the vastus lateralis, the proportion of slow fibers was increased in OW ($p < 0.05$) and decreased in ONW in comparison to YG ($p < 0.05$) (**Figures 1C,D**).

To assess the impact of aging on muscle fibers, we analyzed the myonuclear density and the volume of the myonuclear domain in single muscle fibers isolated from the biopsy samples of vastus lateralis of the three groups of subjects. The results, reported in **Figure 3**, indicate distinct variations in the fibers of the oldest-old subjects in relation to their ability to walk. When compared to young, fibers from ONW subjects exhibited a significant increase ($p < 0.05$) in myonuclei number per unit of length and per unit of volume, while the corresponding reduction in nuclear domain size did not reach statistical significance. Minor and not significant variations in the opposite directions were observed in OW compared to YG.

The functional characterization of the leg extensor muscles was based on the determination of twitch response induced by electrical stimulation, as illustrated in **Figure 2** and reported in **Table 3**. The force parameters (twitch peak, MRFD and MRFR) were reduced in OW and ONW in direct relation to the reduced muscle mass. The rates of force development and relaxation (MRFD/peak and MRFR/peak) were significantly reduced in both OW and ONW compared to YG subjects ($p < 0.05$). Further significant prolongation of the time to peak was detectable in ONW compared to OW ($p < 0.05$).

Quantitative Expression Analysis of Genes Affecting Muscle Growth

To unravel the mechanisms behind the preservation of skeletal muscle fiber thickness in the elderly, we explored the mRNA expression levels of key genes involved in the control of protein synthesis and degradation by quantitative real-time PCR (**Figure 4**). IGF-1 mRNA total expression and the expression of the two IGF-1 isoforms (IGF-1EA, IGF-1EC or MGF) were determined in the vastus lateralis muscle of young and oldest-old subjects. The comparison between YG and both OW and ONW indicated a decreased expression of total IGF-1 in the oldest-old people ($p < 0.01$). Regarding the expression of muscle specific isoforms, IGF-1EA and MGF were almost unaffected in OW compared to YG. The comparison between OW and ONW indicated a clear and significant decrease in expression of both IGF-1EA and MGF in ONW. Next, we determined level of expression of myostatin (also called GDF-8). The myostatin mRNA levels were similarly reduced in the vastus lateralis muscle of both OW and ONW ($p < 0.05$). We also determined the level of expression of another member of TGF-β family, GDF-11, which has been related to the control of muscle mass. No variation of GDF-11 expression was found in both OW and ONW in comparison to YG (**Figure 4**).

The intracellular signaling pathway which links IGF-1 binding to the receptor to an increased protein synthesis in the ribosome is based on three major kinases: phosphoinositide-3-kinase (PI3K), AKT/Protein Kinase B (Akt/PKB) and mammalian target of rapamycin (mTOR). We assessed the expression of AKT1, AKT2, and mTOR in vastus lateralis muscle, and we found a decrease in the level of AKT1 mRNA expression, which reached statistical significance only in the muscles of OW ($p < 0.01$) (**Figure 5**).

Protein Degradation Pathways

We analyzed genes coding for the muscle specific atrophy-related ubiquitin ligases Atrogin-1 and MuRF-1 (**Figures 6A,B**). The expression of both genes was down regulated in the vastus lateralis muscle of both OW ($p < 0.05$ and $p < 0.01$) and ONW ($p < 0.01$ for both genes) compared to the YG, but there was no modification in their expression between OW and ONW.

The expression of the autophagy-related gene LC3 was slightly, but not significantly, modulated in the vastus lateralis muscle of both OW and ONW compared to YG and with no significant difference between the two groups of the oldest-old (**Figure 6C**).

We then determined the expression of transcriptional factor Foxo3A and observed a trend toward values being lower in the

TABLE 3 | Parameters of the electrical evoked twitch response.

	Twitch peak Nm	Time to peak ms	MRFD Nm s^{-1}	MRFR Nm s^{-1}	MRFD/peak s^{-1}	MRFR/peak s^{-1}
YG	46.1 ± 3.1	85 ± 5	879 ± 20	181 ± 8	19.2 ± 1.6	3.9 ± 0.3
OW	27.5 ± 4.0*	94 ± 3*	461 ± 66*	70 ± 12*	17.0 ± 3.2	2.6 ± 0.7*
ONW	18.8 ± 3.98*$	106 ± 5*$	366 ± 37*$	49 ± 9*$	20.1 ± 4.2	2.7 ± 0.9*

*Twitch peak amplitude in Nm, time to peak in ms, maximal rate of force development between 10 and 90% of twitch peak (MRFD in Nm s^{-1}), maximal relaxation rate between 90 and 10% of twitch peak (MRFR in Nm s^{-1}), MFRD and MRFR relative to twitch peak (MRFD/peak; MRFR/peak). The latter two parameters are pure rates, with no relationship to force development. (YG n = 8, OW n = 6, and ONW n = 9). Data are presented as mean ± S.E; *Different from YG ($p < 0.05$); $ Different from OW ($p < 0.05$).*

OW and ONW than in the YG, although the variations did not reach statistical significance (**Figure 6D**).

Energy Sensing Factors

Among the factors involved in energy sensing and mitochondrial biogenesis regulation, we analyzed the gene expression of PGC-1α, the master gene controlling mitochondrial biogenesis, and two metabolic sensors, SIRT-1, AMPKα2, which act upstream of PGC-1α. No differences were found of SIRT-1 expression in the vastus lateralis muscle of the YG, OW, or the ONW (**Figure 7**). The level of AMPKα2 expression was determined to be significantly decreased only in ONW group ($p < 0.05$, **Figure 7**). The level of PGC-1α expression was determined to be decreased ($p < 0.01$) in the vastus lateralis muscle of oldest-old, both the OW and ONW in comparison to the YG (**Figure 7**).

DISCUSSION

The loss of independent mobility becomes a very common condition with advanced age. Thus, for many of the oldest-old (i.e., people 85 years of age or greater, see e.g., (Zizza et al., 2009), the effects of disuse combine or overlap with the impact of aging

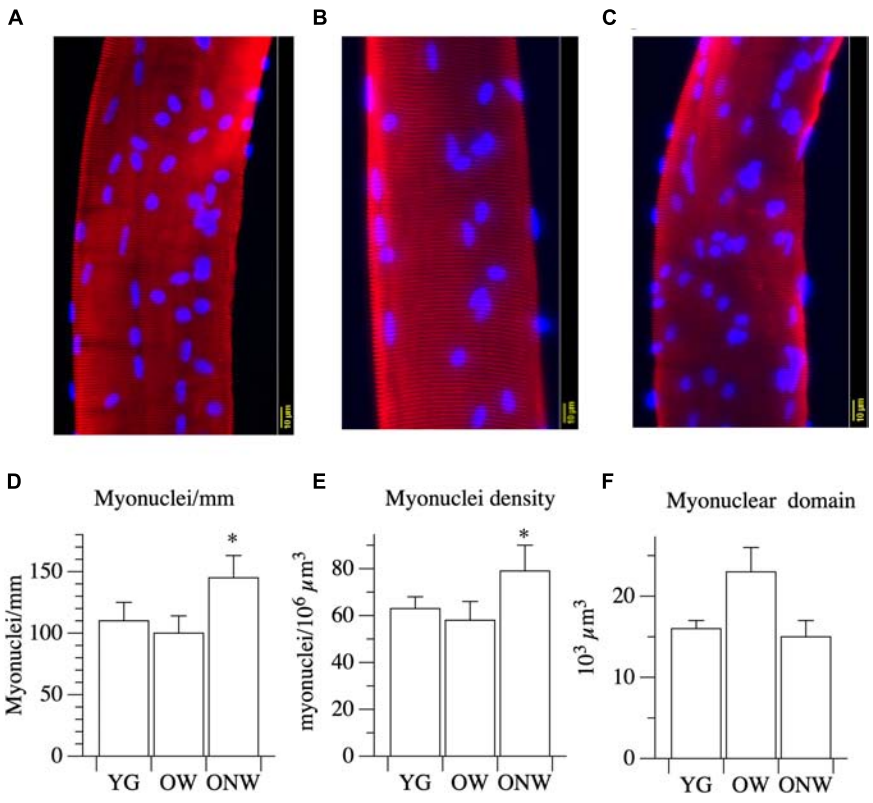

FIGURE 3 | Myonuclear density and myonuclear domain size determined in single muscle fibers from young (YG), walking oldest-old (OW), and non-walking oldest-old (ONW). **(A–C)** typical examples of segment of single fibers stained for nuclear visualization: YG **(A)**, OW **(B)** and ONW **(C)**, **(D)** longitudinal density expressed as number of nuclei per mm of fiber, **(E)** nuclear density, expressed as number of myonuclei per million of cubic micrometers, **(F)** nuclear domain size expressed in cubic micrometers. Data are presented as mean ± S.E.; *Significantly different from YG.

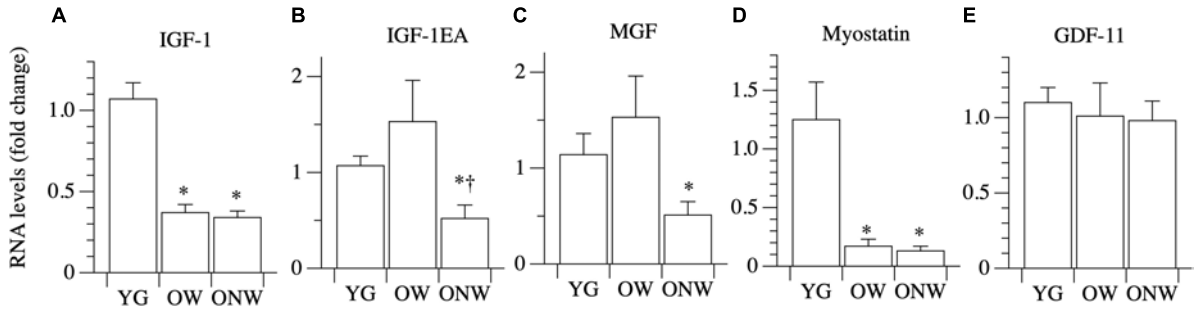

FIGURE 4 | Real time PCR of genes coding for factors affecting muscle growth in young (YG), walking oldest-old (OW), and non-walking oldest-old (ONW). **(A)** IGF-1, **(B)** IGF-1EA, **(C)** MGF or IGF-1EC, **(D)** Myostatin, **(E)** GDF-11. The mRNA levels of the indicated genes were determined by RT-qPCR on muscle obtained by biopsies from YG, OW, and ONW. mRNA levels are expressed as fold change compared to the levels in the vastus lateralis samples of the young (YG n = 8; OW n = 6 ONW n = 9). Data are presented as mean ± S.E.; * Significantly different from YG subjects; †Significantly different from OM subjects.

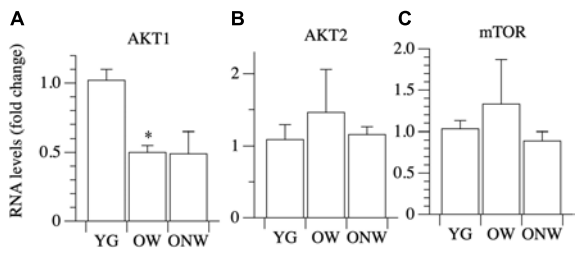

FIGURE 5 | Real time PCR of genes coding for factors involved in protein synthesis in young (YG), walking oldest-old (OW), and non-walking oldest-old (ONW). **(A)** AKT1, **(B)** AKT2, **(C)** mTOR. The mRNA levels of the indicated genes were determined by RT-qPCR on muscle obtained by biopsies from YG, OW, and ONW. mRNA levels are expressed as fold change compared to the levels in the vastus lateralis samples of the young (YG n = 8; OW n = 6 ONW n = 9). Data are presented as mean ± S.E.; *Significantly different from YG subjects.

itself. Evidence published by our team (Venturelli et al., 2015) and others (Raue et al., 2009) documents a dissociation between muscle impairment and atrophy and single muscle fiber size and contractile properties in the oldest-old. In this study, we aimed to elucidate the expression of genes responsible for regulating skeletal muscle fiber size in the lower limbs of the oldest-old still able to walk (OW) and the oldest-old confined to a wheelchair (ONW), each in comparison with young, healthy, individuals (YG). As anticipated (Venturelli et al., 2015), muscle atrophy was evident in both of the oldest-old groups compared to YG, but was more pronounced in ONW than OW, whereas average fiber size was preserved in both oldest-old groups. The present results, however, revealed that, despite similar fiber size, the impact of the loss of the ability to walk was detectable in fiber type distribution, myonuclear density, and gene expression. Actually, the gene expression analysis documented that, in the muscle of the oldest-old, the expression of the positive regulator of protein synthesis IGF-1 was attenuated and so too was the negative regulator myostatin. Coupled with no change in some IGF-1 isoforms, IGF-IEA and IGF-1EC/MGF, and the attenuation of genes responsible for protein degradation (Atrogin, Murf-1, FoXO3 and LC3), the emerging picture is suggestive of a somewhat preserved balance between pro- and anti-atrophy factors.

The determination of thigh muscle mass confirmed the marked age-related skeletal muscle atrophy which was more pronounced in the subjects who were confined to a wheelchair. This loss of muscle mass was accompanied by an approximately proportional decrease in muscle strength, determined *in vivo* with a single electrically stimulated twitch. When the effect of advanced age on the muscle cells was assessed by measuring the cross-sectional area of single fibers, there was no significant difference between the average values obtained in YG and either of the oldest-old groups in agreement with previous findings by our group and others (Raue et al., 2009; Venturelli et al., 2015; Grosicki et al., 2016). This mismatch implies that, at least in the oldest-old, the age-related atrophy is due to the loss of muscle fibers rather than a decrease in fiber size. Here it is worth noting that the immunohistochemistry analysis revealed two very different fiber type distributions as the consequence of advanced aging (OW, predominantly type I or slow fibers) and advanced aging and disuse (ONW, predominantly type II or fast fibers). It should also be recognized that in the skeletal muscle of elderly subjects, the precise identification of fiber type is often uncertain as the proportion of hybrid slow-fast fibers increases significantly (Klitgaard et al., 1990; Rowan et al., 2012). Thus, employing simple fiber counting after immunohistochemical staining with anti-myosin antibodies, hybrid fibers will be considered either slow or fast, depending on the proportion of the myosin isoform expressed. However, the changes observed in the current study seem to provide clear evidence of a fiber type shift. An additional difference in the fibers of the subjects confined to the wheelchair compared to the subjects still able to walk and, this time, also compared to the young, was the increased nuclear density. A similar increase in myonuclei number and density with aging has previously been reported in murine (Brack et al., 2005) and human (Cristea et al., 2010) muscles. It is not clear whether this change is a cause or an effect of fiber atrophy with aging. However, as the number of myonuclei increases in proportion to fiber size during fiber growth, but does not decrease when fibers return the original size, the high nuclear density is suggestive of atrophy (Gundersen and Bruusgaard, 2008). This observation

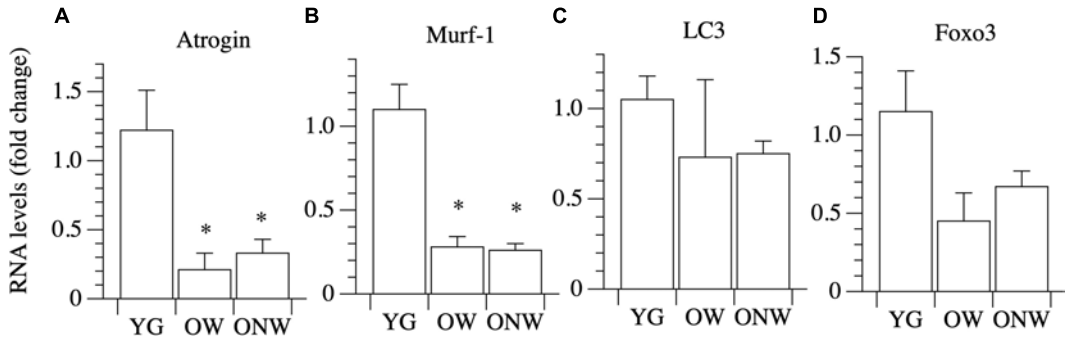

FIGURE 6 | Real Time PCR of genes coding for factors involved in protein degradation in young (YG), walking oldest-old (OW), and non-walking oldest-old (ONW). **(A)** Atrogin, **(B)** Murf-1, **(C)** LC3, **(D)** Foxo3. The mRNA levels of the indicated genes were determined by RT-qPCR on muscle obtained by biopsies from YG, OW, and ONW. mRNA levels are expressed as fold change compared to the levels in the vastus lateralis samples of the young. (YG *n* = 8; OW *n* = 6; ONW *n* = 9). Data are presented as mean ± S.E.; * Significantly different from YG subjects.

helps to explain why, despite exhibiting a predominance of type II fibers, which are generally larger than type I fibers, the type II fibers of the ONW actually tended to be smaller. However, to reiterate, despite these difference in muscle phenotype and muscle size, there was still no significant difference in the average fiber size between the YG and either of the oldest-old groups.

Although skeletal muscle is exposed to numerous factors that may affect skeletal muscle fiber size, including both circulating IGF-1, which has been documented to decrease with age (Perrini et al., 2010), and circulating myostatin, which has been determined to increase with age, at least in elderly women (Bergen et al., 2015), perhaps the most significant factors are expressed and released by the muscle fibers themselves. Specifically, fibers express pro-atrophic and pro-hypertrophic factors, which act in paracrine manner on the muscle. To investigate, at the molecular level, why, with advanced age, the whole muscle is atrophied, but single muscle fiber size is preserved, this study focused on the major paracrine-like factors involved in the regulation of muscle fiber size, including IGF-1, and its two isoforms IGF-1EA and IGF-1EC/MGF, myostatin, and GDF-11. The real time PCR analyses revealed that, compared to the YG, the OW exhibited significantly attenuated expression of total IGF-1 and myostatin, but there was no difference in the expression of IGF-1EA, MGF and GDF-11. These data are intriguing because they suggest that the age-dependent atrophy of the whole muscle is not immediately explained by a tipping of the balance between pro-atrophy and pro-hypertrophy signals, as the decrease in the expression of IGF-1 was balanced by a concomitant decrease in the expression of myostatin. The decrease of IGF-1 expression, reported here, is in agreement with previous observations from skeletal muscle biopsies in old people, which documented a 25–45% age-related decrease in IGF-1 mRNA gene expression (Welle et al., 2002; Leger et al., 2008). In contrast to the present findings, however, previous studies documented either elevated myostatin mRNA expression (Leger et al., 2008) or no change (Welle et al., 2002) as a consequence of aging. Indeed, this study appears to be the first to clearly document a decrease in myostatin in skeletal muscle with aging, in the oldest-old, who with an average age close to 90 years,

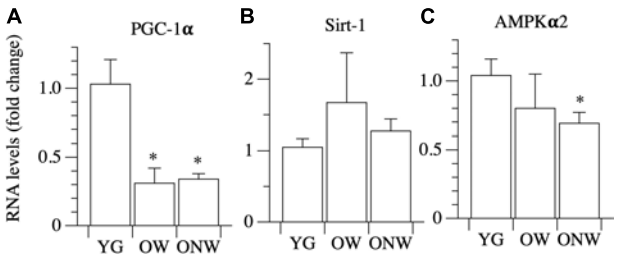

FIGURE 7 | Real time PCR of genes coding for energy sensing factors in young (YG), walking oldest-old (OW), and non-walking oldest-old (ONW). **(A)** PGC-1α, **(B)** Sirt-1, **(C)** AMPKα2. The mRNA levels of the indicated genes were determined by RT-qPCR on muscle obtained by biopsies from YG, OW, and ONW. mRNA levels are expressed as fold change compared to the levels in the vastus lateralis samples of the young (YG *n* = 8; OW *n* = 6; ONW *n* = 9). Data are presented as mean ± S.E.; *Significantly different from YG subjects.

are 15–20 years older than the subjects assessed in the majority of other aging studies. Furthermore, likely also counteracting the fall in both IGF-1 and myostatin, the expression of the two specific IGF-1 isoforms was not attenuated in OW. This suggests a transcriptional re-organization, which may also contribute to the maintenance of muscle fiber size. Of note, in the ONW, the oldest-old group with the most atrophied whole muscle due to the combination of both advanced age and inactivity, both IGF-1EA and MGF were significantly attenuated, but still average muscle fiber size was unchanged. Finally, the oldest-old did not exhibit changes in expression of GDF-11, which has been reported to increase with age in rat muscle and in human serum, an increase which has been related to muscle atrophy (Sinha et al., 2014; Egerman et al., 2015). Thus, unchanged GDF-11 gene expression coupled with a fall in both IGF-1 and myostatin, in addition to unchanged IGF-1 isoforms in the OW, may explain, at least in part, the preservation of muscle fiber size in the oldest-old.

We found that the level of expression of two important effectors of the IGF-1 signaling pathway, such as AKT2 and mTOR, are not modified by aging, whereas AKT1 expression is decreased. While AKT2 is mainly involved in glucose

metabolism, AKT1 is part of the protein synthesis pathway. AKT regulation, however, is mainly evoked at the post-translational phosphorylative level. Furthermore, the direct target of AKT inhibitory action, Foxo3, is down regulated in the vastus lateralis of the oldest-old subjects as well as important genes controlled by Foxo3 and involved in muscle atrophy, such as Atrogin and MURF-1. These data suggest that the down-regulation of factors which trigger the degradation of the protein in muscle cells could play a pivotal role in maintaining the integrity of the skeletal muscle fiber in the oldest-old. Additionally, there was a trend for LC3 (also called MAP1LC3A) gene expression to decrease in vastus lateralis muscle of the oldest-old, although this did not achieve statistical significance. LC3 codes for a protein involved in autophagosome vesicle assembly and can be considered a marker of autophagy. It is worthy of note that, although directed to protein degradation, the mechanism of autophagy is essential to preserve muscle fiber size, as demonstrated by the genetic manipulation of the genes coding for the essential component of the autophagic vesicle (Masiero et al., 2009; Carnio et al., 2014; Jiao and Demontis, 2017).

The current results indicate that the average size of a single muscle fiber is not modified by aging or use, but the histotype of the fibers is significantly modified. In fact, the proportion of slow fibers in the vastus lateralis muscle of OW was increased compared to the corresponding proportion in the YG. In contrast, the number of slow fibers declined in the muscle of ONW. The increase in the number slow fibers in the OW could be either a consequence of a transition from fast-to-slow fibers as a consequence of aging or, as an alternative, may be the result of a specific loss of type II, fast glycolytic, fibers which are most vulnerable to the effects of aging in contrast to the more resistant type I slow oxidative fibers (Anderson and Prolla, 2009). Following a similar line of reasoning, the increase of fast fibers in ONW could be the result of disuse which can cause a slow-to-fast fiber transition [see for a review Blaauw et al. (2013)]. To address this issue, we analyzed the expression of two factors which are implicated in the fast-to-slow and glycolytic-oxidative transitions: SIRT1 and PGC1-α (Chalkiadaki et al., 2014). The mRNA level of SIRT1, a protein deacetylase, correlated with the fiber type transition as it was slightly, but not significantly, increased in OW oldest-old subjects compared to YG. In contrast, the mRNA level of PGC1-α, a protein involved in mitochondrial biogenesis, was dramatically reduced in both groups of oldest-old subjects as already reported in previous aging studies in humans and murine models (Chan and Arany, 2014). Interestingly, PGC1-α levels were not modified by differences in muscle activity for locomotion, suggesting that PGC1-α expression is more connected with the status of the aged skeletal muscle than with a fast-to-slow fiber transition. Therefore, on the whole, these findings support the hypothesis that the decrease of muscle mass observed with aging is due to a loss of fast fibers rather than an increase of the percentage of slow fibers. The combined impact of disuse and aging, however, might lead to a slow-to-fast fiber transition, as occurs in other conditions of muscle disuse and unloading (Schiaffino and Reggiani, 2011; Blaauw et al., 2013).

AMP-activated protein kinase (AMPK) is a key energy-sensitive enzyme that controls numerous metabolic and cellular processes. Previous studies showed a decrease in AMPKα2 activity in vastus lateralis muscle and lower levels of AMPK phosphorylation in the elderly (Li et al., 2012). In the present study, there was evidence of a slight decrease of AMPKα2 mRNA expression in the vastus lateralis of the oldest-old, more evident and statistically significant in ONW than in OW. This observation is in agreement with the finding, in a previous study, that AMPKγ3 subunit protein content was lower in the immobilized leg than in the contralateral leg of older men (Vigelso et al., 2016), suggesting an impaired glucose utilization with aging, which is exacerbated by the lack of movement (Vigelso et al., 2016).

In conclusion, this study confirmed the preservation of the average muscle fiber size in the oldest-old, whether able to walk or confined to a wheelchair, and provided initial insight into the cellular signal network involved in this preservation. In contrast to the commonly documented tipping of the balance in favor of pro-atrophy factors in older adults (decrease in IGF-1 and increases in both myostatin and atrogenes), in the oldest-old the pro-atrophy factors appear to be down-regulated, allowing a partial recovery of the proteostasis balance. Interestingly, the impact of a preserved capacity for locomotion is rather limited, detectable in the fiber type distribution and in the expression of few genes, but with no effect on single fiber cross sectional area. It remains to be established whether the preservation of single fiber cross sectional area is a compensatory response to the age-related loss of motor units and muscle fibers or whether this is a specific response from a sub-population of fibers which are not only able to survive the widespread motoneuron and fiber death, but also to preserve their trophic features. Additionally, the failure of the muscle fibers of the oldest-old to increase in thickness with resistance training (Raue et al., 2009) and the lack of significant difference between walking and not-walking subjects observed in this study, support the view that, in this specific group of old people, the preservation of fibers size is a consequence of a selective process and not of the adaptative response to the progressive loss of motor units. This conclusion implies that the benefit of physical activity in this extreme population is limited, at least at the muscle fiber level.

AUTHOR CONTRIBUTIONS

FN, MV, RR, FS, and CR conceived and designed the study. FN, MV, LM, LT, EM, JZ, RR, FS, and CR performed the experiments. FN, MV, LM, LT, EM, JZ, CM, and CR analyzed the data. FN, MV, LT, RR, CM, and CR interpreted the results. MV, LT, JZ, and CR prepared the figures. FN, MV, RR, FS, and CR drafted the manuscript. MV, RR, and CR edited the manuscript.

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frontiers in Physiology

CORRECTION
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Corrigendum: Skeletal Muscle Fiber Size and Gene Expression in the Oldest-Old With Differing Degrees of Mobility

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A Corrigendum on

Skeletal Muscle Fiber Size and Gene Expression in the Oldest-Old With Differing Degrees of Mobility

by Naro, F., Venturelli, M., Monaco, L., Toniolo, L., Muti, E., Milanese, C., et al. (2019). Front. Physiol. 10:313. doi: 10.3389/fphys.2019.00313

In the original article, there was an error in citing a paper concerning the impact of physical activity on motoneuron survival: Dranseika et al. (2016) was cited instead of Piasecki et al. (2016). A correction has been made to second paragraph of the Introduction, which reads as follows:

The conundrum of greatly diminished muscle size and function, while individual muscle fiber size and function are preserved, may potentially be explained by a loss of muscle fibers. In this respect, the neural system plays a pivotal role. Initially, with progressive motoneuron death and fiber denervation, and, then, by the disappearance of the denervated fibers or, possibly, by partial reinnervation of the surviving fibers by sprouting of slow motoneurons (Delbono, 2003, 2011; Payne and Delbono, 2004; Aagaard et al., 2010; Reid et al., 2012; Venturelli et al., 2018). Interestingly, it is still debated whether the loss of motoneurons can be slowed down by regular physical activity [see Power et al. (2010) in favor and Piasecki et al. (2016) against this view]. Unfortunately, the direct assessment of the impact of neural events on muscle fiber size and number during advanced age and disuse is somewhat complicated (Doherty et al., 1993). However, the comparison between the force developed during maximal voluntary contraction (MVC) and electrically stimulated contraction helps to estimate the contribution of reduced neural drive to muscle deconditioning (Venturelli et al., 2015). Furthermore, the evaluation of *in vivo* single twitch kinetics may further contribute to understand the functional condition of skeletal muscle, as the maximal rates of force development are clearly different among slow and fast motor units (Mero et al., 1991). Unfortunately, information regarding single twitch kinetics in the oldest-old is sparse.

The authors apologize for this error and state that this does not change the scientific conclusions of the article in any way. The original article has been updated.

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The Effect of the Stretch-Shortening Cycle in the Force–Velocity Relationship and Its Association With Physical Function in Older Adults With COPD

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This study aimed to evaluate the effect of the stretch-shortening cycle (SSC) on different portions of the force–velocity (F–V) relationship in older adults with and without chronic obstructive pulmonary disease (COPD), and to assess its association with physical function. The participants were 26 older adults with COPD (79 ± 7 years old; FEV_1 = 53 ± 36% of predicted) and 10 physically active non-COPD (77 ± 4 years old) older adults. The F–V relationship was evaluated in the leg press exercise during a purely concentric muscle action and compared with that following an eccentric muscle action at 10% intervals of maximal unloaded shortening velocity (V_0). Vastus lateralis (VL) muscle thickness, pennation angle (PA), and fascicle length (FL) were assessed by ultrasound. Habitual gait speed was measured over a 4-m distance. COPD subjects exhibited lower physical function and concentric maximal muscle power (P_{max}) values compared with the non-COPD group (both $p < 0.05$). The SSC increased force and power values among COPD participants at 0–100 and 1–100% of V_0, respectively, while the same was observed among non-COPD participants only at 40–90 and 30–90% of V_0, respectively (all $p < 0.05$). The SSC induced greater improvements in force, but not power, among COPD compared with non-COPD subjects between 50 and 70% of V_0 (all $p < 0.05$). Thus, between-group differences in muscle power were not statistically significant after the inclusion of the SSC ($p > 0.05$). The SSC-induced potentiation at 50–100% of V_0 was negatively associated with physical function (r = −0.40–0.50), while that observed at 80–100% of V_0 was negatively associated with VL muscle thickness and PA (r = −0.43–0.52) (all $p < 0.05$). In conclusion, older adults with COPD showed a higher SSC-induced potentiation compared with non-COPD subjects, which eliminated between-group differences in muscle power when performing SSC muscle

actions. The SSC-induced potentiation was associated with lower physical function, VL muscle thickness, and VL PA values. The SSC-induced potentiation may help as a compensatory mechanism in those older subjects with a decreased ability to produce force/power during purely concentric muscle actions.

Keywords: aging, muscle power, resistance training, concentric, eccentric, potentiation

INTRODUCTION

In the last few decades, both life expectancy and the number of years of living with disability have increased among older adults (Crimmins et al., 2016). This increasing proportion of people over 65 years old could lead to a higher incidence of chronic degenerative diseases and to a greater demand for health and social care with a consequent impact on health spending (Lopreite and Mauro, 2017).

Muscle power is recognized as a major target for resistance training interventions conducted to improve physical function in older adults (Sayers, 2008; Reid and Fielding, 2012; Izquierdo and Cadore, 2014). The capacity to produce muscle power is mediated by the ability to exert force and velocity, and thus it depends on the individual force–velocity (F–V) relationship. The evaluation of the F–V profile in older people has been shown to be a novel and effective strategy to differentiate between subjects with different functional states (Alcazar et al., 2018), and to individualize exercise programs in order to improve physical performance and enhance muscle power production in young people (Samozino et al., 2014; Jimenez-Reyes et al., 2016). The F–V profile is commonly evaluated by recording the velocity exerted with increasing isotonic loads or force produced at varying isokinetic velocities (Alcazar et al., 2017). Both methods provide information about the neuromuscular status of the subjects being measured; however, isotonic recordings may be preferred in older people because of their similarity to the movements they perform in daily life. The evaluation of the F–V relationship has been shown to be reliable in older people (Alcazar et al., 2017). This method to assess neuromuscular function in older populations might provide us with several advantages over other methods [such as the one repetition maximum (1RM) test] in terms of relevant information on the force and velocity components of muscle power production (Alcazar et al., 2018).

The stretch-shortening cycle (SSC) is a phenomenon characterized by the enhancement of power production in a concentric muscle action preceded by active muscle stretching (eccentric muscle action) in comparison with the same concentric action preceded by a resting state. The SSC can improve muscle power production by 50% in some tasks such as vertical jumping (Bosco et al., 1982b). The known enhancement of performance due to the inclusion of the SSC has been attributed to the storage of elastic energy in the muscle during the stretch and its re-use as mechanical work during the concentric phase when it follows immediately (Bosco et al., 1982b). Reflexively induced neural input has also been suggested to be involved in the enhanced muscle function derived from a SSC action (Komi and Bosco, 1978; Bosco et al., 1982a; Komi, 1984).

However, while the SSC has been proven to increase neuromuscular performance in young adults (Komi, 1984; Jiménez-Reyes et al., 2014; Pallarés et al., 2014), some discrepancies exist regarding older people. In the aged population, a SSC-related increment in mechanical performance has been reported in some studies (Svantesson and Grimby, 1995; Lindle et al., 1997), although it has also been found to be diminished in comparison with younger subjects (Bosco and Komi, 1980). Interestingly, Izquierdo et al. (1999) found a decreased performance on the bench press exercise after a SSC compared to a solely concentric action in middle-aged and older men, although a greater jump height was achieved after the inclusion of the SSC. Another study observed a significant increase in jump height induced by the SSC in young subjects, but not in the middle-aged and older groups (Paasuke et al., 2003). By contrast, a SSC-induced potentiation was observed during plantar flexions in middle-aged and elderly subjects, without any age-related difference in comparison with young subjects (Svantesson and Grimby, 1995). The discrepancies across studies might be caused by the different muscles being evaluated (e.g., upper vs. lower limbs) and/or the differences among the aged populations being included (e.g., active vs. sedentary older adults). However, there are no studies evaluating the functional relevance that SSC-induced potentiation has in older people. Furthermore, limb muscle dysfunction is a prevalent condition in older people with chronic obstructive pulmonary disease (COPD), and is characterized by the presence of a decreased proportion of type I muscle fibers and oxidative capacity, and reduced muscle cross-sectional area, strength, and endurance (Maltais et al., 2014). The evaluation of the influence of the SSC on muscle performance in these patients might contribute to the understanding of this COPD-related myopathy.

Thus, our main goals were to assess the ability of the SSC to enhance muscle power during concentric actions in older people with COPD, and to evaluate the association between SSC-induced power potentiation and physical function and the influence of muscle size and architecture in older people with COPD.

MATERIALS AND METHODS

Subjects

Twenty-six outpatients with COPD (FEV1 = 53.4 ± 35.6% of predicted; BODE index = 3.9 ± 2.1) and 10 physically active non-COPD older adults participated in this investigation (**Table 1**). COPD subjects had been previously diagnosed by a pulmonologist and were clinically stable. Participants were screened if they were aged ≥ 65 years, community dwelling,

TABLE 1 | Comparison of main characteristics of the study participants.

	COPD group	Control group	
	Mean ± *SD*	**Mean ± *SD***	***p***
Age (years)	78.8 ± 7.1	76.6 ± 4.1	0.159
BMI ($kg \cdot m^{-2}$)	30.7 ± 6.0	30.1 ± 4.0	0.751
SPPB score	10.3 ± 2.1	11.8 ± 0.4	< **0.001**
Habitual gait speed ($m \cdot s^{-1}$)	1.0 ± 0.3	1.2 ± 0.4	**0.022**
VL muscle thickness (cm)	1.84 ± 0.39	1.86 ± 0.21	0.877
VL pennation angle (°)	12.7 ± 4.5	11.8 ± 1.1	0.364
VL fascicle length (cm)	9.9 ± 4.1	9.1 ± 1.0	0.139

BMI, body mass index; SPPB, short physical performance battery; VL, vastus lateralis. Bold values indicate significant differences between groups ($p < 0.05$).

and reported no participation in a regular resistance training program in the previous 6 months. The subjects completed a medical history questionnaire and performed the short physical performance battery (SPPB) (Guralnik et al., 1994) to assess their physical function. Exclusion criteria included a SPPB score < 4, severe cognitive impairment [mini-mental state examination (MMSE) score < 20], neuromuscular or joint injury, stroke, myocardial infarction, or bone fracture in the previous 6 months, uncontrolled hypertension (>200/110 mmHg), or terminal illness. In addition, non-COPD subjects reported regular participation ($\geq$3 days $week^{-1}$) in exercise activities such as walking, cycling, swimming, or running. All the subjects gave their informed consent and the study was performed in accordance with the Helsinki Declaration and approved by the Ethical Committee of the Toledo Hospital (Spain).

Measurements

Physical function was assessed by means of the 4-m habitual gait speed test. After the cue "ready, set, go!" the subjects walked at their habitual gait speed and the time taken to walk 4 m was recorded. The subjects were asked to walk slightly farther than 4 m in order to avoid deceleration just before covering the 4-m distance.

Vastus lateralis (VL) muscle thickness and muscle architecture were assessed using B-mode ultrasonography (MyLab 25, Esaote Biomedica, Genova, Italy), with a 50 mm, 10–15 MHz, linear-array probe (**Figure 1**). Resting ultrasound images were taken at the midpoint of the distance between the superior border of the greater trochanter and the inferior border of the lateral epicondyle of the right leg, with the participant lying on his/her back and the knee slightly flexed at 150° (full knee extension is 180°). The transducer was aligned in the fascicle plane to be able to visualize an optimal portion of fascicles on the ultrasound screen. Then, the images were analyzed by means of image analysis software (ImageJ 1.51q8, NIH, Bethesda, MD, United States). Muscle thickness was measured as the average of the perpendicular distance between the superficial and deep aponeuroses of the VL muscle at three different points on the image (left border, midpoint, and right border). VL pennation

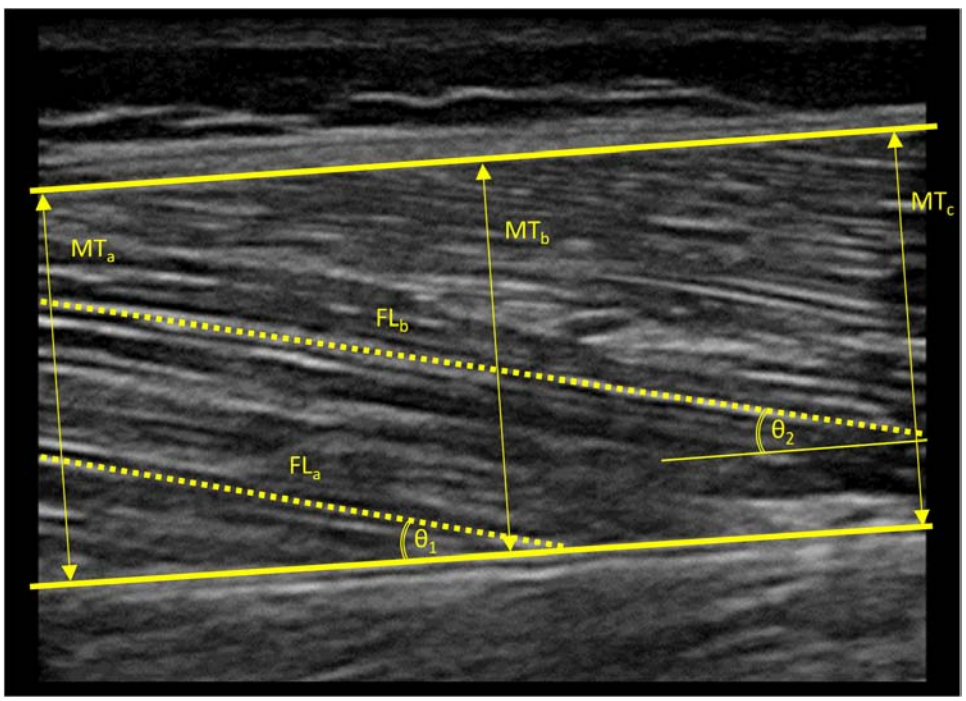

FIGURE 1 | Ultrasound image obtained from the vastus lateralis muscle of a standard subject. Thick lines indicate the superior and inferior aponeuroses of the vastus lateralis muscle. Arrows indicate the three different points at which muscle thickness was measured. Dashed lines indicate the visible portions of muscle fascicles. Arched lines indicate angles formed between the visible muscle fascicles and the inferior aponeurosis. Non-visible portions of muscle fascicles were estimated using a linear extrapolation of fibers and aponeuroses in order to estimate fascicle length. MT, muscle thickness; FL, fascicle length; θ, pennation angle.

angle (PA) and fascicle length (FL) were measured from the visible portion of two fascicles within the same image. In some cases, a small portion of the fascicle extended off the ultrasound window and it was necessary to estimate this non-visible portion using a linear extrapolation of fibers and aponeuroses (Erskine et al., 2009). All the images were collected and digitally analyzed by the same operator. Minimal pressure was applied to the skin during ultrasound assessments and an optimal amount of ultrasound coupling gel was used to ensure image quality. Test–retest coefficients of variation (CV) for muscle architecture measures in our laboratory have previously been reported: 1.9–3.6% (Alegre et al., 2014).

The F–V relationship was assessed in all the subjects after attending two separate familiarization sessions with the leg press equipment (Element+, Technogym, Italy). Then, after a 5-min warm up on a cycle-ergometer (Ergoline, 800 S, Bitz, Germany) and a specific warm up (3 × 10 repetitions at 40% of body mass), the subjects performed two sets of two repetitions with increasing loads (10-kg increments) starting from 40% of their body mass. When the subjects failed to lift a certain load, it was decreased by 5 kg until the 1RM was achieved. Force and velocity data of each repetition were recorded with a linear position transducer (T-Force System, Ergotech, Spain) and a linear regression equation was fitted to the data by means of an Excel® spreadsheet as previously reported (Alcazar et al., 2017). Resting periods between sets were allowed based on the velocity of the preceding repetition (>0.50 m · s^{-1}: 60 s of recovery time; 0.30–0.50 m · s^{-1}: 90 s of recovery time; <0.30 m · s^{-1}: 120 s of recovery time). These rest intervals were adequate to allow the older subjects to perform the second and third repetitions of each set without fatigue, since the highest mean velocity was most frequently exerted during the second repetition (50%), followed by the first repetition (36%) and the third repetition (14%; only performed when necessary, see Alcazar et al., 2017). All subjects were verbally encouraged during testing and all the repetitions were performed as fast and strongly as possible. Mean and instantaneous force and velocity values were obtained in order to conduct the data analysis. Test–retest CV of this F–V testing protocol has been reported to be 4.4–8.5% (Alcazar et al., 2017).

Data Analysis

Mean force and velocity values from the first repetition of each set were recorded to build the only-concentric F–V relationship, since they were preceded by a resting state. Mean force and velocity values from the second repetition of each set were recorded to build the eccentric–concentric F–V relationship, since they were preceded by an active lengthening of muscle. In both cases, force at zero velocity or the force-intercept (F_0), velocity at zero force or the velocity-intercept (V_0), and maximal muscle power (P_{max}) were obtained from the F–V regression equations (Alcazar et al., 2017). Using this procedure, we also obtained force and power values exerted at different contraction velocities (as a percentage of concentric V_0). SSC-induced force/power potentiation at varying contraction velocities was calculated as the relative difference between only-concentric and eccentric–concentric force/power values at the same absolute contraction velocities. In addition, to elucidate the possible mechanisms contributing to SSC-induced power potentiation (a force-related mechanism vs. a velocity-related mechanism), instantaneous force, velocity, and power values across the range of movement during an only-concentric and an eccentric–concentric repetition were analyzed at a low intensity (41.2 ± 10.5% of 1RM) and at a high intensity (81.5 ± 7.2% of 1RM).

Statistical Analysis

All data were examined for normality of distribution with the Shapiro–Wilk's test, and log-transformed in case of a non-uniformity result. Standard descriptive statistics were used for continuous variables. The main characteristics and muscle architecture measures were compared between COPD and non-COPD participants by Student's *t*-tests for independent samples. Significant condition (concentric vs. eccentric–concentric) × group (COPD vs. non-COPD) interactions in SSC-induced potentiation at different intervals of concentric V_0 were assessed by two-way ANOVA tests. When sphericity was violated, the Greenhouse–Geisser correction was applied. Pairwise comparisons were performed using the Bonferroni test. In addition, within-group differences between instantaneous force, velocity, and power values obtained from a concentric and an eccentric–concentric repetition were assessed with Student's *t*-tests for dependent samples. Pearson's correlation was used to assess the association of SSC-induced power potentiation with physical function, muscle thickness, and muscle architecture. Statistical analyses were performed using SPSS v20 (SPSS Inc., Chicago, IL, United States) and the alpha level was set at 0.05.

RESULTS

No differences in age or body mass index (BMI) were found between COPD and non-COPD older subjects ($p > 0.05$) (**Table 1**). As expected, non-COPD subjects showed increased SPPB scores and habitual gait speed values compared with the COPD group (both $p < 0.05$). No between-group differences were reported in terms of VL muscle architecture ($p > 0.05$).

The concentric and eccentric–concentric F–V and power–velocity (P–V) relationships of the participants are displayed in **Figure 2**. No between-group differences were found in terms of concentric F_0 values (COPD: 1949.4 ± 621.2 N vs. non-COPD: 1834.9 ± 736.0 N; $p > 0.05$), while non-COPD participants showed greater concentric V_0 (0.86 ± 0.36 m · s^{-1}) and P_{max} (379.2 ± 200.1 W) values compared with the COPD group (0.54 ± 0.15 m · s^{-1} and 267.7 ± 120.4 W, respectively) (both $p < 0.05$). In contrast, no between-group differences were observed in terms of eccentric–concentric F_0 (COPD: 2045.8 ± 610.4 N and non-COPD: 1876.4 ± 687.1 N) or P_{max} (COPD: 323.6 ± 143.7 W and non-COPD: 412.9 ± 194.4 W) values (both $p > 0.05$), while significant differences existed for eccentric–concentric V_0 values (COPD: 0.63 ± 0.21 m · s^{-1} and non-COPD: 0.90 ± 0.35 m · s^{-1}; $p < 0.01$).

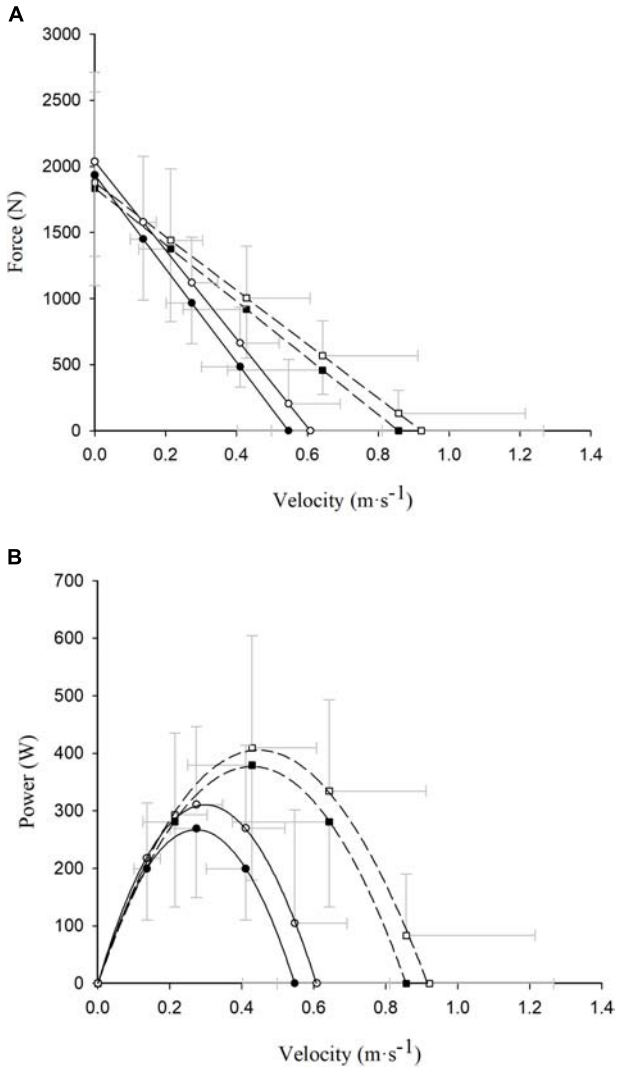

FIGURE 2 | Read figures from up to down. Differences between concentric (closed symbols) and eccentric–concentric (open symbols) force **(A)** and power **(B)** values exerted at different absolute contraction velocities in COPD (circle and continuous line) and healthy older (square and dashed line) participants. Data reported as mean ± *SD*.

SSC-Induced Potentiation in the F–V Relationship

Significant condition × group interactions were observed at 50, 60, and 70% of V_0 for the F–V relationship (all $p < 0.05$) (**Table 2**). Pairwise comparisons showed that the SSC increased force values compared with only-concentric repetitions at all 10% intervals of V_0 (i.e., 0–100% of V_0) among COPD subjects (all $p < 0.05$), while the same was observed among non-COPD older participants only between 40 and 90% of V_0 (all $p < 0.05$). No significant differences were reported between COPD and non-COPD subjects regarding force production at the different V_0 intervals for any test condition (i.e., concentric and eccentric–concentric) ($p > 0.05$).

SSC-Induced Potentiation in the P–V Relationship

No significant condition × group interactions were reported at the different V_0 intervals for the P–V relationship ($p > 0.05$) (**Table 3**). However, the SSC led to increased power values compared with only-concentric repetitions at all 10% intervals of V_0 (except at 0% of V_0) among COPD participants, and between 30 and 90% of V_0 among non-COPD participants (all $p < 0.05$). On the other hand, significant differences between COPD and non-COPD older subjects were noted in the concentric P–V relationship between 1 and 99% of V_0 (all $p < 0.05$), while between-group differences in the eccentric–concentric P–V relationship were only noted at 1% of V_0 ($p < 0.05$).

Force-Related vs. Velocity-Related SSC-Induced Potentiation in Muscle Power

Loads equivalent to 41.2 ± 10.5% (0.675 ± 0.145 s of CON duration) and 81.5 ± 7.2% (0.914 ± 0.249 s of CON duration) of 1RM were selected as representative of the high-force/low-velocity and low-force/high-velocity regions of the F–V relationship, respectively. Differences were observed in terms of force production at different points on the range of movement between the concentric and the eccentric–concentric repetitions (**Figures 3A,B**). While these differences seemed to be located in the mid-portion (40–90%) of the range of movement with the low load (i.e., ∼40% of 1RM), they were distributed slightly more toward the beginning of the movement (20 and 40–80%) in the high-load condition (i.e., ∼80% of 1RM) (all $p < 0.05$). Velocity (**Figures 3C,D**) and power (**Figures 3E,F**) differences between the concentric and the eccentric–concentric repetitions also reached statistical significance for both the low- and the high-load conditions between 20 and 100% of range of movement (all $p < 0.05$). These differences were similarly distributed along the range of movement at both intensities, except at the beginning of the movement (0–10%), where significant differences were not observed in any case ($p > 0.05$).

Association Between SSC-Induced Potentiation and Other Functionally Relevant Variables

Habitual gait speed showed a negative association with SSC-induced power potentiation among COPD participants. Specifically, the associations reached statistical significance at 50, 60, 70, 80, and 90% of V_0 ($r = -0.40$ to -0.50; all $p < 0.05$). A negative association was also observed between VL muscle thickness and SSC-induced power potentiation at 80, 90, and 99% of V_0 ($r = -0.44$ to -0.49; all $p < 0.05$). Furthermore VL PA was found to be negatively associated with SSC-induced power potentiation at 70, 80, 90, and 99% of V_0 ($r = -0.43$ to -0.54; all $p < 0.05$). Finally, no significant correlations were found between VL FL and SSC-induced power potentiation.

TABLE 2 | Comparison of concentric and eccentric–concentric force values at various contraction velocities relative to maximal concentric unloaded shortening velocity.

	COPD group		Control group		ANOVA interaction
	CON	SSC	CON	SSC	
V_0 (%)	Mean ± *SD*	Mean ± *SD*	Mean ± *SD*	Mean ± SD	
0	1949.4 ± 621.2	2045.8 ± 610.4*	1834.9 ± 736.0	1876.4 ± 687.1	0.483
1	1929.9 ± 614.9	2027.8 ± 604.6*	1816.5 ± 728.6	1859.0 ± 681.1	0.472
10	1754.4 ± 559.0	1865.4 ± 552.1*	1651.4 ± 662.4	1701.9 ± 627.3	0.363
20	1559.5 ± 496.9	1685.1 ± 494.9*	1467.9 ± 588.8	1527.3 ± 567.9	0.235
30	1364.6 ± 434.8	1504.7 ± 439.1*	1284.4 ± 515.2	1352.7 ± 509.2	0.123
40	1169.6 ± 372.7	1324.4 ± 385.6*	1100.9 ± 441.6	1178.1 ± 451.3*	0.055
50	974.7 ± 310.6	1144.1 ± 335.3*	917.4 ± 368.0	1003.5 ± 394.5*	**0.030**
60	779.8 ± 248.5	963.7 ± 289.9*	734.0 ± 294.4	828.9 ± 339.5*	**0.030**
70	584.8 ± 186.3	783.4 ± 252.0*	550.5 ± 220.8	654.3 ± 287.4*	**0.048**
80	389.9 ± 124.2	603.0 ± 225.6*	367.0 ± 147.2	479.8 ± 239.8*	0.080
90	194.9 ± 62.1	422.7 ± 214.8*	183.5 ± 73.6	305.2 ± 200.2*	0.120
99	19.5 ± 6.2	260.4 ± 218.6*	18.3 ± 7.4	148.0 ± 175.9	0.159
100	0 ± 0	242 ± 221*	0.0 ± 0.0	130.6 ± 174.1	0.163

*CON, concentric; SSC, eccentric–concentric; V_0, maximal unloaded shortening velocity; SD, standard deviation.*Significant differences compared with CON values ($p < 0.05$). Bold values indicate a significant ANOVA interaction ($p < 0.05$).*

TABLE 3 | Comparison of concentric and eccentric–concentric power values at various contraction velocities relative to maximal concentric unloaded shortening velocity.

	COPD group		Control group		ANOVA interaction
	CON	SSC	CON	SSC	
V_0 (%)	Mean ± *SD*	Mean ± *SD*	Mean ± *SD*	Mean ± SD	
0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	–
1	10.6 ± 4.8	11.1 ± 4.7*	15.0 ± 7.9¥	15.4 ± 7.6¥	0.788
10	96.4 ± 43.3	102.1 ± 43.5*	136.5 ± 72.0¥	140.6 ± 69.1	0.688
20	171.3 ± 77.1	184.4 ± 78.2*	242.7 ± 128.0¥	251.9 ± 123.0	0.545
30	224.9 ± 101.1	246.9 ± 104.4*	318.6 ± 168.0¥	333.7 ± 161.9*	0.376
40	257.0 ± 115.6	289.8 ± 122.5*	364.1 ± 192.1¥	386.1 ± 186.0*	0.228
50	267.7 ± 120.4	312.8 ± 133.1*	379.2 ± 200.1¥	409.1 ± 195.3*	0.159
60	257.0 ± 115.6	316.2 ± 137.3*	364.1 ± 192.1¥	402.7 ± 190.6*	0.164
70	224.9 ± 101.1	299.8 ± 136.8*	318.6 ± 168.0¥	367.0 ± 172.7*	0.206
80	171.3 ± 77.1	263.6 ± 134.8*	242.7 ± 128.0¥	301.8 ± 144.6*	0.258
90	96.4 ± 43.3	207.7 ± 136.4*	136.5 ± 72.0¥	207.2 ± 114.5*	0.307
99	10.6 ± 4.8	140.5 ± 146.3*	15.0 ± 7.9¥	96.9 ± 105.7	0.345
100	0.0 ± 0.0	132.1 ± 148.1*	0.0 ± 0.0	83.2 ± 107.0	0.349

*CON, concentric; SSC, eccentric–concentric; V_0, maximal unloaded shortening velocity; SD, standard deviation.*Significant differences compared with CON values ($p < 0.05$). ¥Significant differences compared with the COPD group ($p < 0.05$).*

DISCUSSION

Our main findings were: (1) older people with COPD showed lower levels of concentric P_{max} compared with age-matched physically active older adults without COPD; (2) COPD participants exhibited a greater SSC-induced potentiation in force values compared with non-COPD subjects; (3) between-group differences in P_{max} disappeared after the inclusion of a SSC muscle action; and (4) the SSC-induced potentiation was negatively associated with physical function, muscle thickness, and PA among COPD older subjects.

Limb muscle dysfunction is a systemic consequence of COPD characterized by the presence of a decreased proportion of type I muscle fibers (Gosker et al., 2007) and oxidative capacity (Green et al., 2008a), and reduced muscle cross-sectional area, maximal strength, and endurance (Couillard et al., 2002, 2003). In addition, we observed a 22% lower concentric P_{max} in COPD compared with non-COPD older subjects, which was fundamentally caused by a deficit in the velocity component of muscle power or the ability to produce force at relatively high contraction velocities. This fact contrasts with a particular characteristic of limb muscle dysfunction in

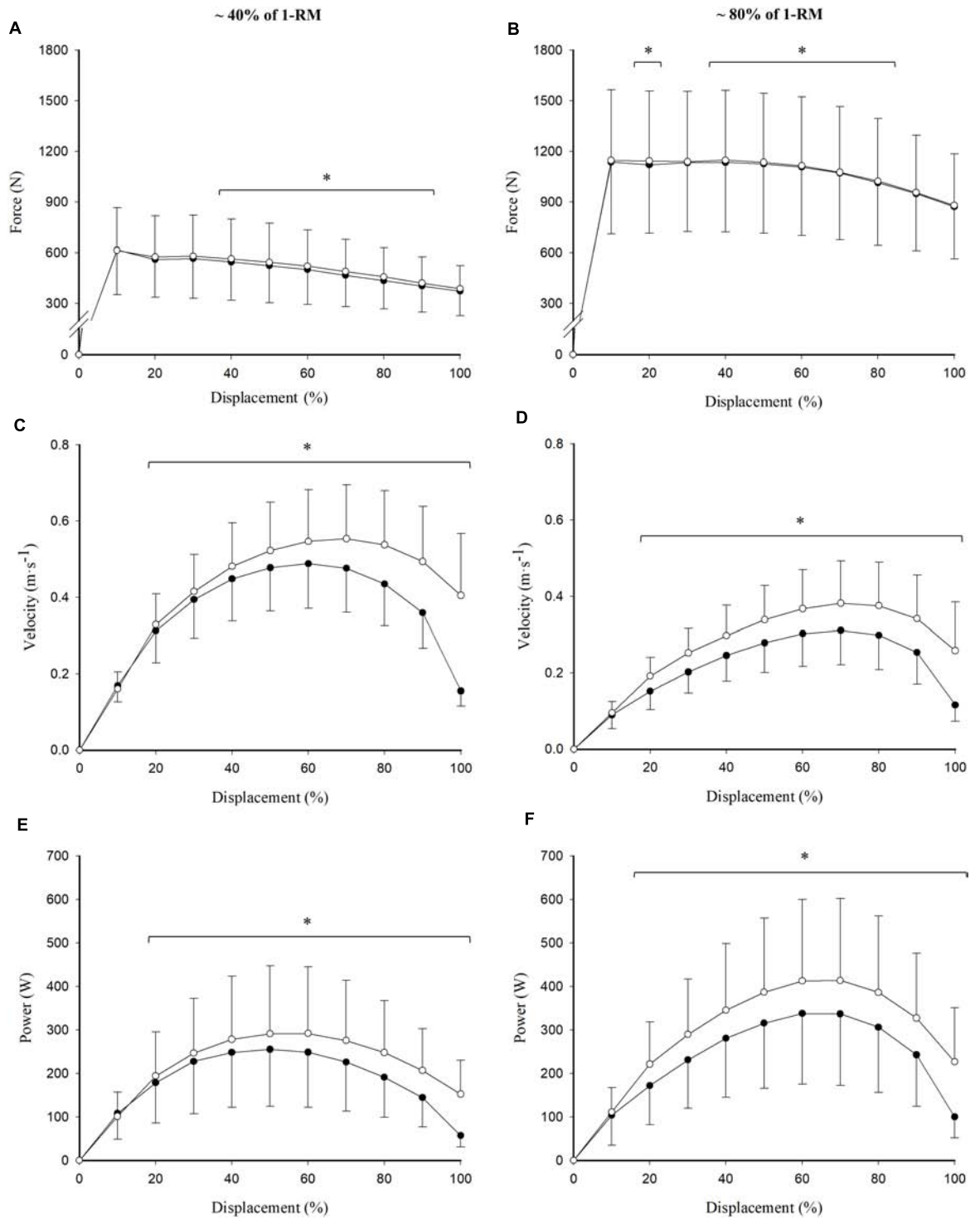

FIGURE 3 | Read figures from up to down and from left to right. Differences between concentric (closed circles) and eccentric–concentric (open circles) force **(A,B)**, velocity **(C,D)**, and power **(E,F)** values across the range of movement at a low (41.2 ± 10.5% of 1RM; **A,C,E**) and a high (81.5 ± 7.2% of 1RM; **B,D,F**) intensity. Data reported as mean ± standard deviation. *Significant differences between concentric and eccentric–concentric values ($p < 0.05$).

COPD, in which in contrast with normal aging, people with COPD experience a shift in fiber type distribution from type I fibers to type II fibers (Gosker et al., 2007). However, single muscle fibers of COPD patients may present altered excitation–contraction coupling (Green et al., 2008b) and decreased ATPase activity compared with age-matched healthy controls

(Stubbings et al., 2008), contributing to the slowing-down of the F–V relationship observed in the present investigation.

On the other hand, the inclusion of the SSC within repetitions led to greater improvements in force in COPD compared with non-COPD older subjects, especially among those contraction velocities eliciting the highest power values in the eccentric–concentric F–V relationship (i.e., 50–70% of concentric V_0). This fact partially compensated previous differences in concentric P_{max}, so that differences in eccentric–concentric P_{max} did not reach the statistical significance. Nevertheless, a relatively moderate variability in the rate of SSC-induced potentiation was observed among subjects (**Figure 4**). A higher variability in the effect induced by the SSC in older adults in comparison with younger subjects has previously been reported (Bosco and Komi, 1980). This variability may be explained by the different mechanisms involved in the SSC-induced potentiation that have been suggested in the literature: increased neural drive, tendon recoil, and contractile potentiation (Cohen, 1988; Hopkins et al., 2009).

Although increased muscle excitation levels have been observed during the 200 ms before the start of the concentric phase of a SSC action compared with a purely concentric one (Walshe et al., 1998), there is evidence showing a similar neural drive to contracting muscles during the concentric phase of a purely concentric and a SSC muscle action (Walshe et al., 1998; Finni et al., 2003). However, other different mechanisms might explain the SSC-induced force increment observed during a concentric muscle action. Tendons are visco-elastic structures that exhibit both rate-dependent (viscous) and rate-independent (elastic) properties. During a SSC action, tendons undergo initial lengthening in the early eccentric phase, with smaller changes in length in the late eccentric phase (Earp et al., 2017). Additional lengthening is experienced by tendons in the early concentric phase until skeletal muscle force peaks and tendons reach their peak length (Earp et al., 2017). After that, tendons start shortening and release elastic energy (Finni et al., 2003; Earp et al., 2017), that can be as high as 85% of that stored during previous lengthening (Shadwick, 1990). Thus, the contribution of tendon shortening to muscle-tendon complex velocity has been reported to be 50% in some cases, although this proportion varied among subjects (Chino et al., 2008). Because of this mechanism of tendon recoil, the contraction velocity of muscle fascicles is reduced for a given muscle-tendon unit velocity (Finni et al., 2003). Therefore, muscle fascicles can produce a greater amount of force at a corresponding joint velocity according to the F–V relationship of skeletal muscles. This mechanism might be enhanced by other structures within muscles that may exhibit spring-like properties (such as actomyosin cross-bridges, actin and myosin filaments, titin, and the connective tissue scaffolding of the extracellular matrix) (Roberts, 2016). In addition, residual force enhancement after stretching has been associated with active and passive muscle contraction-related mechanisms (Rassier and Herzog, 2004). It has been demonstrated that thick filaments are switched on by mechanical stress (Irving, 2017). The increase in the proportion of attached cross-bridges caused by the mechanical stress imposed over the thick filaments (Irving, 2017) and the contributing role of the protein titin to active force production (Herzog et al., 2016) might both explain the increased force production during the early phase of a concentric muscle action preceded by an eccentric muscle action. Furthermore, SSC-induced power potentiation seems to be highly dependent on the fast transition from the eccentric to the concentric muscle action (Newton et al., 1997; Schmidtbleicher, 1997; Lappin et al., 2006). Perhaps this is the reason for the differences observed in the magnitude of the SSC-induced power potentiation values across the F–V relationship, with the effect of the SSC being relatively greater for the fastest muscle actions (i.e., lower contraction times).

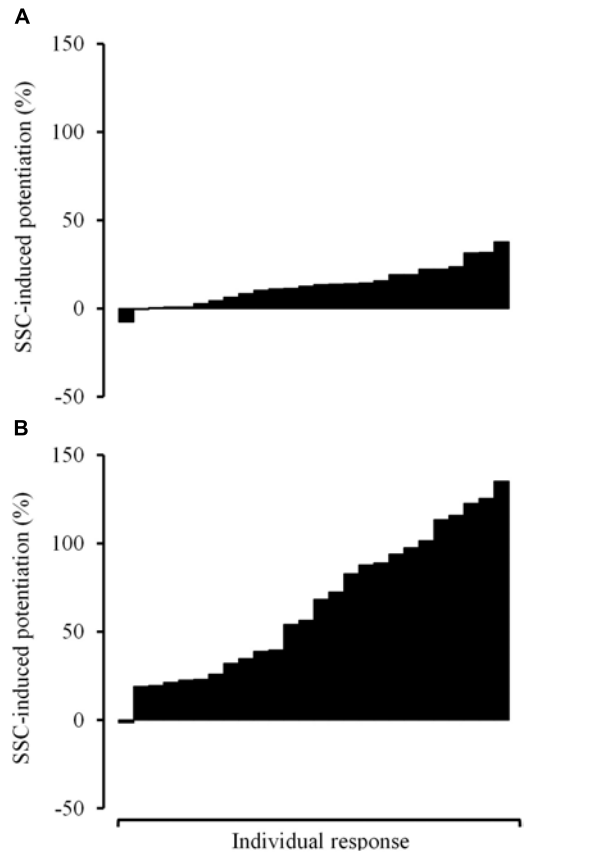

FIGURE 4 | Read figures from up to down. Individual response observed among COPD participants in terms of stretch-shortening cycle-induced potentiation in force values at 20% of V_0 **(A)** and 80% of V_0 **(B)**. V_0, maximal unloaded shortening velocity.

Surprisingly, a higher SSC-induced power potentiation was associated with lower physical function, VL muscle thickness, and VL PAs in our sample of older people with COPD. Moreover, physically active non-COPD older subjects showed higher physical function values and lower SSC-induced potentiation than COPD subjects. In order to confirm these results, we looked at the data obtained in another study previously published by our research group with community-dwelling older adults (Alcazar et al., 2017), and observed a statistically significant negative association between SSC-induced power potentiation at 60% of 1RM and habitual gait speed ($r = -0.43$; $p < 0.05$;

n = 31; unpublished results). Unfortunately, the eccentric–concentric F–V relationship was not assessed in that study, and so we could not include those subjects in the present investigation. Therefore, the SSC-induced power potentiation seems to be a negative outcome in older people. Impaired muscle excitation of the agonist muscles has been reported to be a major cause of mobility limitations in older people (Clark et al., 2010). The longitudinal decrement in muscle excitation levels among older subjects has been observed to be as high as 9% per year, and has been strongly associated with the loss of muscle power with aging (Clark et al., 2013). A possible explanation for the negative association between SSC-induced power potentiation and physical function is that they both share the same characteristic: decreased concentric muscle function. Muscle excitation levels during a solely concentric muscle action are severely impaired in older subjects with mobility limitations, which may also be associated with decreased muscle thickness and PA values. This concentric deficit might be compensated during a SSC muscle action by the effects of tendon recoil and the potentiation of the contractile material within skeletal muscles, and thus the mobility limited older subject would exhibit a greater power generating capacity during the SSC muscle actions compared with the purely concentric muscle actions. Actually, there is strong evidence supporting the specific and strong age-related deficit in maximal concentric force, which is progressively higher at faster contraction velocities (Thom et al., 2007), while maximal eccentric force values seem to be better preserved with age (Roig et al., 2010), especially in COPD individuals (Mathur et al., 2007). In fact, the relatively preserved eccentric force values observed in older people have been associated with enhanced residual force enhancement after active stretching (Power et al., 2012). Thus, the augmented SSC-induced response might be a compensatory mechanism to produce muscle power in those older subjects with an impaired physical function and reduced muscle size and PA values. This SSC-related compensatory mechanism has also been reported to be involved in the attenuated loss of muscle function observed during countermovement compared with squat jumps after exercise-induced muscle damage in young subjects (Byrne and Eston, 2002).Unfortunately, the tendon properties and muscle excitation levels of the participants were not assessed in the present investigation in order to find the possible mechanisms leading to the enhanced SSC-induced response observed among COPD individuals. However, we should highlight that this is the first study in which the effect of the SSC on the F–V relationship of older people (with and without COPD) has been investigated. Future studies are warranted in order to elucidate the actual mechanisms involved in the SSC-induced power potentiation observed in mobility-limited older subjects with COPD.

CONCLUSION

Older adults with COPD produced lower concentric P_{max} values than non-COPD subjects as a consequence of a diminished capacity to produce force at relatively high contraction velocities. The SSC was found to enhance muscle power production, especially among older subjects with COPD, eliminating differences in eccentric–concentric P_{max} values between COPD and non-COPD participants. Finally, SSC-induced potentiation was negatively associated with physical function, VL muscle thickness, and PA, but not with VL FL. Future studies should be conducted in order to determine the mechanisms that explain a greater SSC-induced potentiation in mobility-limited older adults with COPD.

DATA AVAILABILITY

The datasets for this study will not be made publicly available because we are unable to provide the minimal dataset because these data are a part from a big project that are pending to be published. While the main paper is waiting, you can request any doubt from the data to the corresponding author via e-mail: Luis.Alegre@uclm.es.

AUTHOR CONTRIBUTIONS

RN-C, JA, CR-L, JL-R, AA-A, IA, FG-G, and LA conceived and designed the experiments and interpreted the results of research. RN-C, JA, CR-L, JL-R, and FG-G performed the experiments. RN-C, JA, IA, LA, A-AA, and CR-L analyzed the data.RN and JA drafted manuscript and prepared tables/figures. RN-C, JA, CR-L, JL-R, AA-A, IA, FG-G, and LA edited and critically revised the paper and approved the final version of the manuscript.

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Physical Activity Associated Proteomics of Skeletal Muscle: Being Physically Active in Daily Life May Protect Skeletal Muscle From Aging

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Muscle strength declines with aging and increasing physical activity is the only intervention known to attenuate this decline. In order to adequately investigate both preventive and therapeutic interventions against sarcopenia, a better understanding of the biological changes that are induced by physical activity in skeletal muscle is required. To determine the effect of physical activity on the skeletal muscle proteome, we utilized liquid-chromatography mass spectrometry to obtain quantitative proteomics data on human skeletal muscle biopsies from 60 well-characterized healthy individuals (20–87 years) who reported heterogeneous levels of physical activity (not active, active, moderately active, and highly active). Over 4,000 proteins were quantified, and higher self-reported physical activity was associated with substantial overrepresentation of proteins associated with mitochondria, TCA cycle, structural and contractile muscle, and genome maintenance. Conversely, proteins related to the spliceosome, transcription regulation, immune function, and apoptosis, DNA damage, and senescence were underrepresented with higher self-reported activity. These differences in observed protein expression were related to different levels of physical activity in daily life and not intense competitive exercise. In most instances, differences in protein levels were directly opposite to those reported in the literature observed with aging. These data suggest that being physically active in daily life has strong and biologically detectable beneficial effects on muscle.

Keywords: physical activity, proteomics, skeletal muscle, mitochondria, spliceosome, immunity, DNA damage, mass spectrometry

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INTRODUCTION

The decline in muscle strength is one of the most striking phenotypes of aging, which is only partially accounted for by a reduction in muscle mass, suggesting a loss of cellular and molecular integrity of muscle tissue, and/or impairment of neuromuscular control with aging. Low muscle strength is a powerful, independent predictor of slow gait, mobility disability, and early mortality (Metter et al., 2002; Hicks et al., 2012; Moore et al., 2014). No interventions are currently available that can prevent or attenuate the decline in muscle strength with aging except exercise, especially resistance training. In spite of this evidence, the percentage of people who regularly exercise is

still low and this percentage declines with aging (Sallis, 2000; Milanovic et al., 2013). It has been suggested that people who have an active lifestyle in daily life have a slower decline of muscle mass and strength with aging (Paterson et al., 2007; Cartee et al., 2016). Understanding how physical activity in daily life affects muscle physiology in older persons might help in developing new interventions that, by targeting the same mechanisms triggered by physical activity, could prevent the development of muscle impairment with aging. Numerous studies have investigated the impact of a sedentary lifestyle and low physical activity on health outcomes in both younger and older individuals. Physical inactivity, either long or short-term, negatively affects muscle performance and is associated with diminished aerobic capacity, as well as reduced insulin sensitivity and basal metabolic rate (Biolo et al., 2005; Hamburg et al., 2007; Bogdanis, 2012). Furthermore, physical activity alone has been shown to improve and regulate metabolic homeostasis and metabolic efficiency (Palmnas et al., 2018). Overall, an active lifestyle could be conceptualized as a mixture of aerobic and resistance exercise, but the intermittent, and variable mixture of these activities make it difficult to study (Neufer et al., 2015). Endurance and resistance training elicit both common and specific metabolic/morphologic adaptations in muscle, some of which are common between tissues (Wilkinson et al., 2008; Kazior et al., 2016). In general, the stress that is induced by exercise challenges energy homeostasis in myocytes, shifting the cellular environment towards an oxidative state (Egan and Zierath, 2013). This induces microdamage that stimulates both transcriptional and posttranscriptional responses, which then promotes synthesis of specific proteins that seek to reestablish a different homeostatic equilibrium. Endurance training maximally stimulates mitochondrial biogenesis, enhances aerobic metabolism and fatty acid utilization, and produces change in muscle fiber composition (Kohn et al., 2011; Groennebaek and Vissing, 2017). In contrast, heavy resistance training stimulates the synthesis of contractile proteins, leading to muscle hypertrophy, and increases in maximal contractile force speed and output. Whether an active lifestyle is sufficient to activate the same biological mechanisms triggered by endurance and resistance training is unknown. In 2014, an NIH workshop recommended that -omics be used for "Understanding the Cellular and Molecular Mechanisms of Physical Activity-Induced Health Benefits" (Neufer et al., 2015). Indeed, skeletal muscle adaptation to an active lifestyle should be reflected in specific gene expression and, ultimately, proteomic profiles (Izquierdo et al., 2004; Mahoney et al., 2005; Larina et al., 2014).

In recent years, a handful of studies have examined the protein composition of human muscle cell types and tissues (Hojlund et al., 2008; Gonzalez-Freire et al., 2017) including proteomic differences between old and young muscle (Doran et al., 2009; Gueugneau et al., 2014; Lourenco dos Santos et al., 2015; Murgia et al., 2017), athletes and non-athletes (Abe et al., 2018), exercise in extreme conditions (Larina et al., 2014; Schild et al., 2015), and physical activity and metabolic disorders (Goodpaster et al., 2001; Camera et al., 2017; Kleinert et al., 2018). These studies have helped to characterize the physiological adaptations of healthy human muscle to different types of exercise (Rockl et al., 2007; Trappe et al., 2016). Most of these studies focused on the acute and immediate effects of short bouts of high intensity exercise in either human or mice/rat models (Guelfi et al., 2006; Egan and Zierath, 2013; Petriz et al., 2017), as well as long-term effects of exercise (Crane et al., 2013; Mosole et al., 2014; Belaya et al., 2018). However, very little research has focused on assessing the association of daily physical activity with the muscle proteome in healthy community-dwelling individuals. To verify whether an active lifestyle is associated with detectable changes in skeletal muscle and to start to characterize these changes, we performed a quantitative, mass spectrometry-based proteome analysis of muscle specimens from a group of well-characterized healthy individuals with a wide age-range (20–87 years) and who self-reported different levels of physical activity. Independent of age and technical covariates, we found that high levels of physical activity (versus low levels) were associated with an overrepresentation of mitochondrial proteins, tricarboxylic acid (TCA) cycle enzymes, chaperone proteins, and proteins associated with genome maintenance. In contrast, proteins related to the spliceosome and transcription regulation, immune proteins, apoptosis proteins, DNA damage proteins, and senescent proteins were underrepresented in muscle of participants who reported higher physical activity. Differences observed were mostly opposite to those observed with skeletal muscle aging. Interestingly, only some of these differences contradict observations in the literature that have been associated with aging in skeletal muscle.

MATERIALS AND METHODS

Study Design and Participants

Muscle biopsies analyzed in this study were collected from participants from the genetic and epigenetic study of aging and laboratory testing (GESTALT). Potential participants were enrolled in GESTALT if they met the IDEAL (Insight into the Determination of Exceptional Aging and Longevity) criteria; namely if the participants were free of major diseases, except for controlled hypertension or a history of cancer that had been clinically silent for at least 10 years, were not chronically medicated (except one on antihypertensive medication), had no physical or cognitive impairments, had a BMI less than 30 kg/m^2, and were not professional athletes. Details regarding IDEAL criteria are reported elsewhere and includes information on medical history, physical exams, and blood tests results that were collected and interpreted by a trained nurse practitioner (Schrack et al., 2014). Participants were admitted to 3 days of testing at the Clinical Research Unit of the National Institute on Aging Intramural Research Program. Overall, data and muscle specimens from 60 participants were available for this study. However, two participants were excluded because the muscle specimen provided was too small to obtain reliable proteomic data. Therefore, data from 58 participants dispersed over a wide age-range (20–34 years, n = 13; 35–49 years, n = 11; 50–64 years, n = 12; 65–79 years, n = 12; 80+ years, n = 10) were used for this study. Participants' height and weight were objectively assessed. The GESTALT protocol was approved by

the Intramural Research Program of the US National Institute on Aging and the Institutional Review Board of the National Institute of Environmental Health Sciences. All participants provided written, informed consent at every visit.

Muscle Biopsies

Prior to skeletal muscle biopsies, the depth of the subcutaneous fat (uncompressed and compressed) was determined using MRI images of the middle thigh performed on the previous day. A region above the vastus lateralis muscle was marked at the mid-point of a line drawn between the great trochanter and the mid−patella upper margin. The skin was prepped with povidone – iodine (Betadine®) and ethyl alcohol, and the outside areas covered with sterile drapes. The biopsy site was anesthetized, first intradermally using a 27-gauge needle and then subcutaneously using a 23-gauge × 1 1/2 -inch needle, follow by an 18-gauge spinal needle, with ∼15 mL of 1% lidocaine in sodium bicarbonate. This method ensured that the subcutaneous tissue and muscle fascia, but not the muscle fibers, were infiltrated with the anesthetic so as not to distort the tissue structure. A 6−mm Bergstrom biopsy needle was inserted through the skin and fascia incision into the muscle, and muscle tissue samples were obtained by manual suctioning and cutting with a coaxial blade. The biopsy specimen then was cut into small sections, which were snap frozen in liquid nitrogen and subsequently stored at −80°C until used for analyses.

Physical Activity Assessment

Physical activity participation was determined using an interview-administered standardized questionnaire originally developed for the health, aging, and body composition study (Brach et al., 2004) and modeled after the leisure-time physical activity questionnaire (Taylor et al., 1978). Levels of moderate to vigorous physical activity was estimated from responses to questions concerning brisk walking ("walking at a fast pace where it may be difficult for you to speak normally, sometimes called power walking"), weight or circuit training activities and vigorous exercise activities ("like bicycling, swimming, running, aerobics, basketball, soccer, rowing, racquet sports, stair-stepping, elliptical or cross-country ski machine or exercycle"). Participation data for up to four vigorous activities was collected. For each type of activity, participants were asked whether they performed the activity in the past 2 weeks and, if so, the frequency performed, and average amount of time spent for each activity. Total participation time in moderate to vigorous physical activity per week was calculated by multiplying frequency by amount of time performed for each activity, summing all of the activities then dividing by two to derive minutes of moderate to vigorous physical activity per week. For analyses, the following categories were used: <30 min per week of high intensity physical activity was considered "not active" and coded as 0; high intensity physical activity ≥30 and <75 min was considered "moderately active" and coded as 1, high intensity physical activity ≥75 and <150 min was considered "active" and coded as 2, and high intensity physical activity ≥150 min was considered to "highly active" and coded as 3. The resulting ordinal variable from 0 to 3 was used in the analysis. Participation in casual walking was determined in a similar manner. One-way ANOVA, non-parametric, and chi-square tests (continuous and categorical variables) were used to test for sample differences between physical activity groups and age groups.

Sample Preparation for LC-MS Analysis

Muscle samples were snap frozen in liquid nitrogen immediately after collection and stored at -80°C. On average, 8 mg of muscle tissue was pulverized in liquid nitrogen and lysed [8 M urea, 2 M thiourea, 4% CHAPS, 1% Triton X-100, 50 mM Tris (Sigma), pH 8.5] and sonicated. Protein concentration was determined using a 2-D Quant Kit (GE Healthcare Life Sciences). Detergents and lipids were removed using a methanol/chloroform extraction protocol (Bligh and Dyer, 1959). Proteins were resuspended in urea buffer [8 M urea, 2 M thiourea, 150 mM NaCl (Sigma)], reduced (50 mM DTT), and alkylated (100 mM iodoacetamide), then diluted 12 times with 50 mM ammonium bicarbonate, and digested {18 h at 36°C using a trypsin/LysC mixture [Promega, 1:50 (w/w)]}. Peptides were desalted, speed vacuum dried, and stored at −80°C. Tandem mass tag (TMT) labeling was performed according to the manufacturer's instructions (TMT6plex, Thermo Fisher, Cat# 90066). Donor IDs were blinded and randomized to prevent TMT bias. Each TMT set included one donor from each of the 5 age groups and one reference sample. Each sample was spiked with 200 fM of bacterial beta-galactosidase digest (SCIEX) prior to TMT labeling. Labeled peptides were combined and fractionated.

High-pH RPLC Fractionation and Concatenation Strategy

High-pH RPLC fractionation was performed as described previously (Wang et al., 2011). Skeletal muscle tissues samples were separated using an organic gradient (5–50% B, 100 min) into 99 fractions (1 min each) and merged into 33 master fractions (fraction 1, 34, 67 = master fraction 1, fraction 2, 35, 68 = master fraction 2, and so on). Combined fractions were speed vacuum dried, desalted, and stored at −80°C until final LC-MS/MS analysis.

Nano LC-MS/MS Analyses

Skeletal muscle samples were analyzed using an UltiMate 3000 Nano LC Systems coupled to a Q Exactive HF mass spectrometer (Thermo Scientific, San Jose, CA, United States). Each fraction was separated by reverse phase on a 35-cm capillary column (3-μm C18, Hamilton, HxSil cat 79139) with 150 μm ID at 650 nL/min (5–30% B, 205 min). Tandem mass spectra were obtained using a Q Exactive HF mass spectrometer with a heated capillary temperature +280°C and spray voltage set to 2.5 kV. Full MS1 spectra were acquired from 300 to 1500 m/z at 120,000 resolution and 50-ms maximum accumulation time with automatic gain control set to 3 × 106. Dd-MS2 spectra were acquired using a dynamic m/z range with a fixed first mass of 100 m/z. MS/MS spectra were resolved to 30,000 with 155-ms of maximum accumulation time and the automatic gain control target set to 2 × 105. The 12 most abundant ions were selected for fragmentation using 30% normalized high collision energy.

A dynamic exclusion time of 40 s was used to discriminate against the previously analyzed ions.

Proteomics Informatics

Raw data from each sample fraction were converted to mascot generic format (.mgf) using MSConvert (ProteoWizard 3.0.6002), producing a list of MS ions with retention times and MS/MS spectra. This list of ions was searched with Mascot 2.4.1 and X!Tandem CYCLONE (2010.12.01.1) using SwissProt Human sequences from the Uniprot database (Version Year 2015, 20,200 sequences, appended with 115 contaminants). The search engine uses each protein sequence from the database to produce every possible peptide from it according to the following search parameters: TMT6plex lysine and n-terminus as fixed modifications and variable modifications of carbamidomethyl cysteine, deamidation of asparagine and glutamate, and carbamylation of lysine, the N-terminus, and oxidized methionine. These modifications were frequently set as variable modifications because they are often generated by sample preparation. A peptide mass tolerance of 20 ppm and 0.08 Da were allowed, according to the known mass accuracy of the instrument, for precursor and fragment ions, as well as two missed cleavages. The TMT channels' isotopic purity was corrected according to the TMT Kit instructions.

Results from the Mascot and X!Tandem search engines were analyzed by Scaffold Q+ 4.4.6 (Proteome Software, Inc), and the peptide and protein probabilities were calculated by PeptideProphet and the ProteinProphet probability model (Keller et al., 2002; Nesvizhskii et al., 2003). The data were filtered at thresholds of 0.1% peptide false discovery rate (FDR), 1% protein FDR, and requiring a minimum of 1 unique peptide for protein identification. The experiments in this study compared proteins across multiple samples, identifying quantifiable peptides as only those detected across all samples (n = 58), reducing the probability of false peptide discovery. Finally, proteins were included for analysis even when identified from a single specific peptide, but only if the identification was confirmed by more than one search engine. As for single peptide quantification, the spectrum-to-spectrum variability was no higher between spectra from the same peptide than between spectra from different peptides from the same protein, reducing the likelihood of any differential "bias" in reporter ions from peptide to peptide. More importantly, TMT was taken as a relative, not absolute, quantification. Even if there were such a bias, it would be the same across samples and, thus, the relative quantification would not be affected. Spectral quantitative values were extracted from Scaffold and decoy spectra, while contaminant spectra and peptide spectra shared between more than one protein were removed. Typically, spectra are shared between proteins if the two proteins share most of their sequence, usually for protein isoforms. Spectra were retained for further analyses if they were exclusive to only one protein and were identified in all 6 channels across each TMT set. The log2 transformed spectral abundance was normalized by median subtraction from all reporter ion intensity spectra belonging to a protein across all channels. Relative protein abundance was estimated by the median of all peptides for a protein combined together. Protein sample loading effects from sample preparations were corrected by median polishing, i.e., subtracting the channel median from the relative abundance estimate across all channels to have a zero median (Herbrich et al., 2013; Kammers et al., 2015).

Linear mixed regression models were used to examine the effect of physical activity (considered as an ordinal variable) on each protein after adjusting for age, race, BMI, the ratio between type I (MYH7) and type II myosin fibers (MYH1, MYH2, and MYH4) in order to account for different fiber type composition, and TMT mass spectrometry experiments. The ratio of type I and type II fiber was estimated from the log2 normalized protein intensities of MYH7, MYH1, MYH2, and MYH4. The expression of these fiber types was calculated for each participant and the ratio of the fiber types was estimated as the ratio of MYH7 over the sum of MYH1, MTH2, and MYH4 protein intensity. The size of the beta coefficient for physical activity (as an ordinal variable) was used to quantify the effect of physical activity on each protein independent of covariates, and the significance of the association from the regression model was determined with p-values derived from lmerTest. Any protein $p < 0.05$ was considered to be significant and reported in the main text as a protein associated with physical activity. Multiple testing correction of the p-values were performed using the Benjamini-Hochberg method in R and reported in the supplement. The regression model was performed using R 3.3.4 (R Core Team, 2016) with the lme4 v1.1. library. Proteins with a negative beta coefficient were considered to be negatively associated with physical activity and the proteins with positive beta coefficient as positively associated with physical activity. Protein annotations were performed using GeneOntology, Uniprot keyword, and manual curation. Heat maps and hierarchical cluster analysis were performed using the non-linear minimization package in R (Gaujoux and Seoighe, 2010). GraphPad PRISM 6.07 and R packages were used for statistical analysis and generation of figures. The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE partner repository with the dataset identifier PXD011967 (Vizcaino et al., 2016).

RESULTS AND DISCUSSION

Study Participation and Proteome Isolation From Muscle Biopsies

We sought to determine proteomic differences in the muscle tissues of 20–87 year-old subjects according to physical activity status, while also considering age and sex as covariates. **Supplementary Table S1** details the characteristics of study participants by age group, while **Table 1** contains the distribution of the self-reported frequency of major physical activity tasks according to physical activity groups. Not active participants tended to be younger, but age differences between groups were not statistically significant. On average, participants in each of the four groups were slightly overweight, but BMI did not differ between the physical activity groups. Due to the inclusion criteria, all participants were free of major diseases.

Identification of Physical Activity-Associated Proteins

After adjusting for confounders, of the ∼4,300 proteins identified from the TMT experiments (**Supplementary Table S2**) 1,019 proteins were significantly associated with physical activity ($p < 0.05$) (**Supplementary Table S3**). The beta coefficients for proteins associated with physical activity (indicating log2 fold expression across physical activity levels) ranged from −0.22 (gene CRIP1) to 0.15 (gene PRKAG2), where negative and positive beta coefficients indicated proteins which log2 abundances were lower or higher, respectively, with higher physical activity. Of the 1,019 physical activity-related proteins, 324 and 695 proteins were upregulated and downregulated, respectively (**Figure 1A**). The top proteins that were positively associated with physical activity were mitochondrial proteins involved in energy metabolism or fatty acid oxidation, such as succinate dehydrogenase cytochrome b560 subunit (SDHC), methylglutaconyl-CoA hydratase (AUH), succinate dehydrogenase (SDHB), and isobutyryl-CoA dehydrogenase (ACAD8). SDHC is a complex II protein that declines with age (Melov et al., 2007) and has been reported to increase with physical activity (**Figure 1B**). Isobutyryl-CoA dehydrogenase, which was increased with physical activity in this study, has been reported to be downregulated in obesity in a twin study (Pietilainen et al., 2008).

The top significant protein that was negatively associated with physical activity was kinesin-1 heavy chain (KIF5B, beta = −0.0281, p = 6.61E-07) (**Figure 1B**), which is implicated in the transport of membrane organelles and other cellular cargoes along microtubules and has been associated with axonal outgrowth, neuroplasticity, and neurogenesis. Kinesin-1 also affects mitochondrial morphology by moving mitochondria along microtubule tracks away from the mitochondrial reticulum, leading to mitochondrial fragmentation (Iqbal and Hood, 2014). Wang et al. (2013) used a mouse model with a muscle-conditional knock-out for kinesin-1 to reveal severe muscle dystrophy, suggesting that KIF5b plays significant roles in myofibrillogenesis and in myotendinous junction stability. In linkage family studies, single nucleotide polymorphisms in KIF5B positively regulated the cardiac response to acute training (Argyropoulos et al., 2009). In addition, KIF5B has been recently identified as a novel gene associated with amyotrophic lateral sclerosis (Nicolas et al., 2018). Although this discovery may appear to be counterintuitive, it is consistent with the increased KIF5B gene expression observed in a rat model of sciatic nerve ligation (Kazemi et al., 2016). Thus, our findings suggest that the skeletal muscle from individuals who are physically active have low rates of fiber denervation, which no longer requires KIF5B upregulation to drive reinnervation (Mosole et al., 2014).

Cysteine-rich protein 1 (CRIP1, beta = −0.217, p = 0.0016) was also strongly underrepresented in muscle from persons who were highly physically active. CRIP1 is a zinc-finger protein involved in the cytokine-mediated immune response (Lanningham-Foster et al., 2002) and in cell proliferation (Louis et al., 1997). In a previous study of gene expression in blood peripheral cells, CRIP1 was one of the genes most highly upregulated with aging, possibly as a part of the pro-inflammatory state of aging that tends to be attenuated by physical activity (Nakamura et al., 2012).

The Mitochondrial Proteome Is Upregulated With Higher Physical Activity Independent of Age

In this study, mitochondrial proteins represented roughly 15% of all proteins detected from muscle biopsies. Roughly 40% of all mitochondrial proteins were differentially regulated based on level of self-reported physical activity (**Figure 2A**) and as many as 75% of these proteins were significantly overrepresented with higher physical activity (**Figures 2B,D**). This is consistent with established effects of exercise on mitochondrial biogenesis, suggesting that routine, daily physical activity is sufficient to improve mitochondrial muscle content, and may counteract diminishing oxidative capacity in skeletal muscle that has been previously described with normal aging and is associated with mobility loss (Lanza et al., 2008; Schild et al., 2015; Choi et al., 2016; Deschenes and Chabot, 2017; Robinson et al., 2017). Conversely, downregulated proteins were evenly distributed throughout various functional categories (**Figures 2C,E**).

The majority of the upregulated mitochondrial proteins associated with higher physical activity were from the

TABLE 1 | Baseline characteristics of the GESTALT participants included in this study.

Physical activity group	Not active (0)	Moderately active (1)	Active (2)	Highly active (3)	*p*-value
	(*n* = 11)	(*n* = 8)	(*n* = 14)	(*n* = 25)	
Gender	*M*7, *F*4	*M*6, *F*2	*M*7, *F*7	*M*16, *F*9	0.686
Age, years	50.8 ± 21.6	58.3 ± 25	59.7 ± 18.8	52 ± 19.1	0.596
Race	6*C*, 4*AA*, 1*A*	4*C*, 3*AA*, 1*A*	11*C*, 3*AA*	20*C*, 4*AA*, 1*A*	0.51
BMI, kg/m^2	25.7 ± 2.5	26.6 ± 3.3	25.1 ± 3.1	26.4 ± 3	0.558
†Weight/circuit training	0 ± 0	31 ± 11	77 ± 38	153 ± 194	0.1274
†Vigorous exercise	1 ± 4	4 ± 10	31 ± 42	171 ± 145	<0.0001
†Brisk walking	0 ± 0	29 ± 21	89 ± 117	140 ± 157	0.001
†Casual walking	121 ± 77	78 ± 75	254 ± 178	249 ± 227	0.051

Gender, the number of participants is reported on top of each column; M, male; F, female. Age expressed in years as mean ± standard deviation. Race: C, Caucasian; AA, African American; and A, Asian. Body Mass Index (BMI) expressed as mean ± SD. †Physical activity expressed in minutes/week as mean ± SD. The sample difference between physical activity groups were calculated by One-way ANOVA, non-parametric, and chi-square tests (continuous and categorical variables).

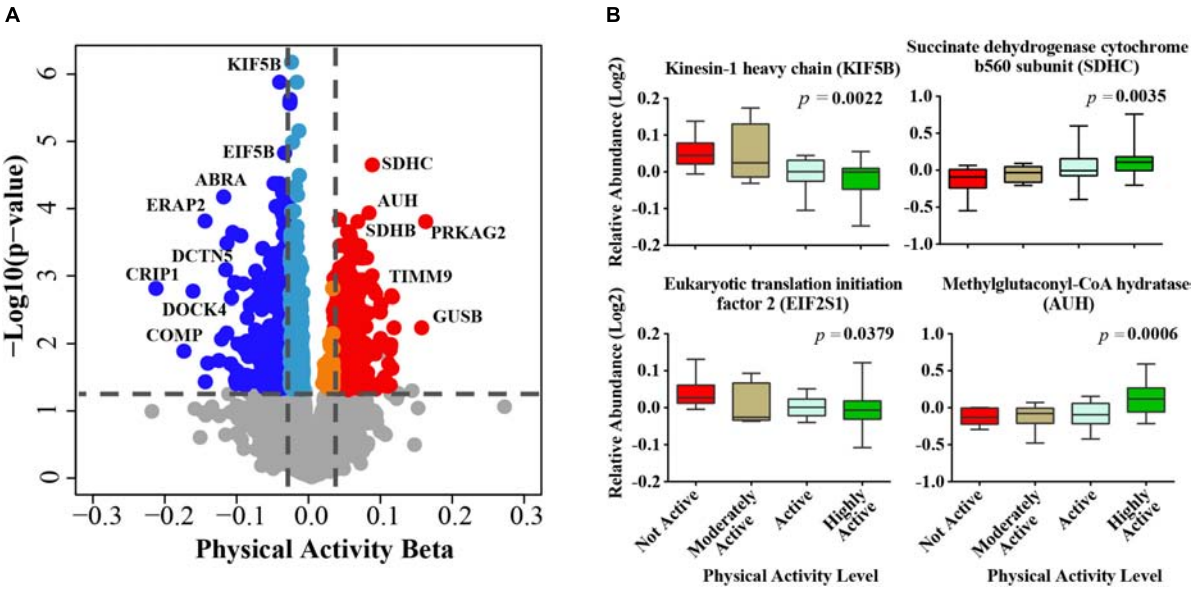

FIGURE 1 | Physical activity associated proteins in human skeletal muscle. **(A)** Proteins associated with physical activity. Level of significance of each protein was assessed by lmerTest from the mixed model regression results and the protein was reported as significant if the $p < 0.05$. Red circles are proteins overrepresented with higher physical activity and blue circles are proteins underrepresented with higher physical activity. The proteins that were least affected by physical activity are shown as gray, light blue, and orange. **(B)** The average raw data protein levels are shown for most significantly overrepresented or underrepresented with higher physical activity, according to self-reported physical activity level. The left axis represents the log2 relative abundance and the *x*-axis the level of physical activity.

mitochondrial inner membrane (36.5%). Both structural proteins and enzymes in the mitochondrial inner membrane play fundamental roles in transducing energy through oxidative phosphorylation to produce ATP (Saraste, 1999; Huttemann et al., 2007). In response to high activity, proteins from the electron-transportation chain (ETC) were upregulated. The top upregulated proteins across the 5 complexes were NADH-dehydrogenase from complex I, SDHB from complex II, cytochrome *c* from complex III, cytochrome *c* oxidase from complex IV, and ATP synthase for complex V (**Figure 3A**). Beyond the ETC protein components, assembly factor proteins for the mitochondrial complex subunits were upregulated, especially those involved in complex I biogenesis (**Figure 3A**). Interestingly, the mitochondrial transcription factor A (TFAM, beta = 0.045, $p = 0.0026$) was also upregulated (beta = 0.045, $p = 0.0026$). This is consistent with the notion that exercise stimulates the activity of peroxisome proliferator-activated receptor gamma co-activator-1 alpha (PGC-1α), which in turn stimulates the transcription of TFAM (Perry and Hawley, 2018). TFAM binds to specific mtDNA sites and promotes the transcription and replication of mtDNA, which ultimately results in mitochondrial biogenesis (Bengtsson et al., 2001).

Mitochondrial carriers are essential for supplying the substrate that is essential for proper ETC function. From this study, 10 mitochondrial solute carrier proteins were overrepresented with physical activity, namely SLC16A1, SLC25A10, MPC2, SLC25A4, SLC25A3, SLC25A20, SLC25A19, SLC25A11, SLC25A12, and SLC25A5. SLC25A4 is particularly interesting, as it is the main energy shuttle that imports ADP from the cytosol and exports ATP produced in the mitochondrial matrix by ATP synthase. Two other important proteins overrepresented with physical activity should also be noted. The first, creatine kinase S-type (CKMT2, beta = 0.054, $p = 0.0297$), has been implicated in transporting high energy phosphate produced by oxidative phosphorylation to the cytosol in muscle cells in the form of phosphocreatine. The second is adenylate kinase 2 (AK2, beta = 0.034, $p = 0.0196$), which catalyzes the formation of ADP from ATP and AMP, a main sensor of cellular energy homeostasis and an essential process in adenine nucleotide metabolism. Energy production in mitochondria through the ETC requires adequate function of the TCA cycle. Fourteen TCA cycle proteins were significantly ($p < 0.05$) upregulated in skeletal muscle with physical activity (**Figure 3B**).

Human sirtuins (SIRTs) are a heterogeneous class of proteins that have ADP-ribosyltransferase, deacetylase or deacylase activity, and these proteins have been implicated in aging, metabolic regulation, mitochondrial biogenesis, and inflammation, leading to them being considered promising therapeutic targets (Bonkowski and Sinclair, 2016). SIRTs require NAD+ and are thereby considered to be sensors of metabolic and energy status (Chang and Guarente, 2014). In this study, SIRT3 (beta = 0.05, $p = 0.0007$) and SIRT5 (beta = 0.037, $p = 0.01$) – both of which are localized to the mitochondria and regulate energy metabolism, playing an important role in regulating mitochondrial biogenesis – were overrepresented in persons with higher physical activity. SIRT3 is the most potent deacetylase among known sirtuins and previous studies have indicated that SIRT3 concentration declines with aging and increases with physical activity (Lanza et al., 2008; Munoz et al., 2018). Recent data revealed that SIRT5 modulates a TCA

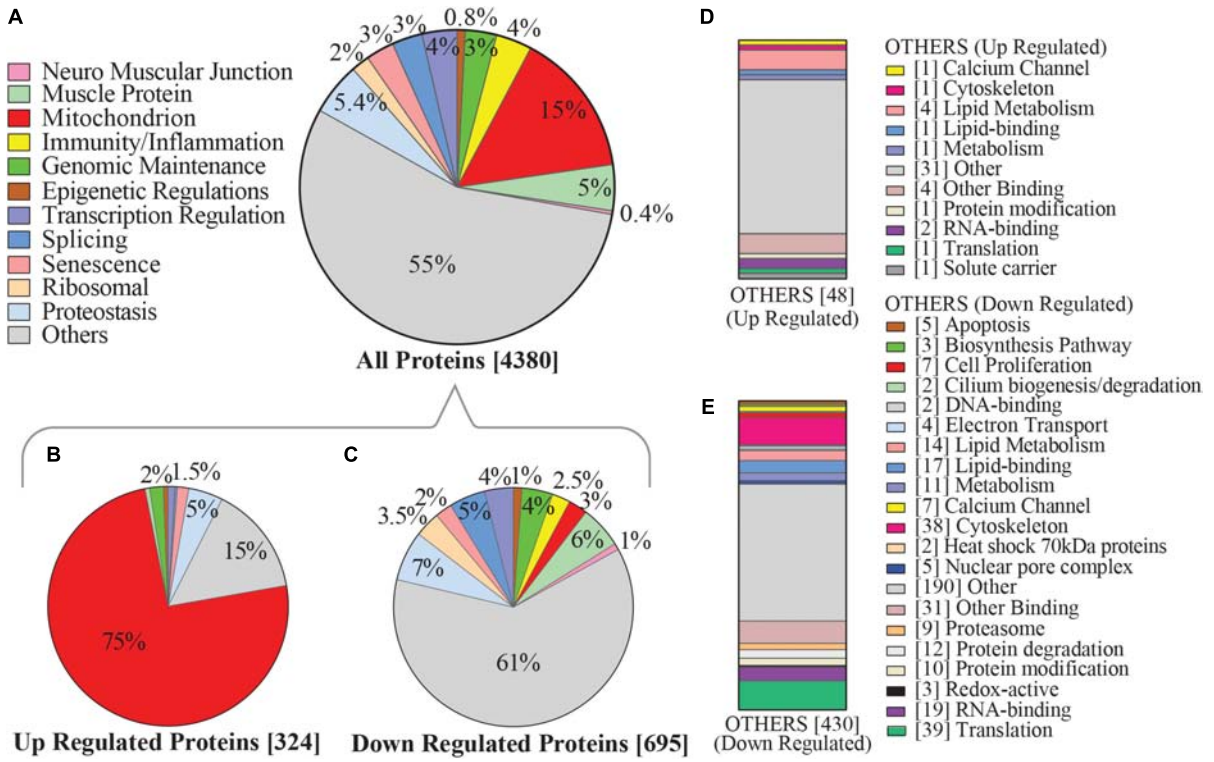

FIGURE 2 | Categorization of physical activity associated hallmarks of aging proteins in human skeletal muscle. **(A)** Categories of proteins which were detected by mass spectrometry are shown, focusing on pathways that emerge from aggregated clusters of proteins and have been associated with aging and/or physical activity. Level of significance of each protein was assessed by lmerTest from the mixed model regression results and the protein was reported as significant if the $p < 0.05$. **(B)** Proteins most significantly upregulated with higher physical activity, of which 75% were mitochondrial proteins. **(C)** 695 proteins were significantly underrepresented with higher physical activity, including those connected with proteostasis, splicing, and immunity/inflammation. **(D)** Subcategorization of the 48 upregulated "others" category proteins from **B**, which were not considered in detail in this study. **(E)** Subcategorization of the downregulated "others" category proteins from **C** not considered in detail in this study, including protein classes associated with cell proliferation, cytoskeleton, lipid metabolism, and translation. Square bracket on left shows number of proteins associated in each category.

cycle intermediate via alternate lysine modifications - including succinylation and malonylation – and modulates enzymes that control fatty acid beta oxidation and the TCA cycle in skeletal muscle fibers (Bringman-Rodenbarger et al., 2018). Overall, our findings demonstrate that high physical activity has a strong effect on mitochondrial proteins, including ETC proteins and TCA cycle regulation proteins, in addition to proteins that contribute to maintaining ETC integrity and function.

Physical Activity Is Associated With Lower Representation of Immune Proteins and Inflammatory Mediators

Intense exercise has a strong effect on immune function; an acute bout of exercise is associated with proinflammatory cytokine production and immunodepression during recovery from fatigue (Peake et al., 2017; Windsor et al., 2018). In contrast, individuals who exercise regularly tend to have enhanced immune function and less evidence of a proinflammatory state (Sellami et al., 2018). In this study, we found that several innate immune proteins linked to macrophage function were negatively associated with physical activity, including macrophage capping protein (CAPG), WD repeat and FYVE domain containing 1 protein (WDFY1), Chitinase domain-containing protein 1 (CHID1), myosin 18A (MYO18A), Inositol polyphosphate phosphatase-like 1 (INPPL1), and Poly(rC)-binding protein 2 (PCBP2) (**Table 2**). Proteins that belong to the complement system, another component of innate immunity, were also downregulated in muscle from participants who reported higher physical activity. These included complement factors C1RL and CFD, as well as complement c1Q binding protein (**Table 2**). Finally, high-mobility group box-1 protein (HMGB1), a redox sensitive protein which promotes leukocyte recruitment to the muscle and activates TLR4 via downstream chemokine and cytokine release, was also underrepresented with higher physical activity (Vezzoli et al., 2010). Overall, regular physical activity in daily life appears to be associated with lower activation of innate immunity response. However, it remains unclear whether the downregulation of innate immunity is direct or mediated by the accumulation of intramuscular adipose tissue in skeletal muscle, which occurs more frequently in sedentary individuals and is notoriously pro-inflammatory (Ferrucci and Fabbri, 2018).

Proteins indicative of adaptive immunity were also underrepresented with higher physical activity. These included

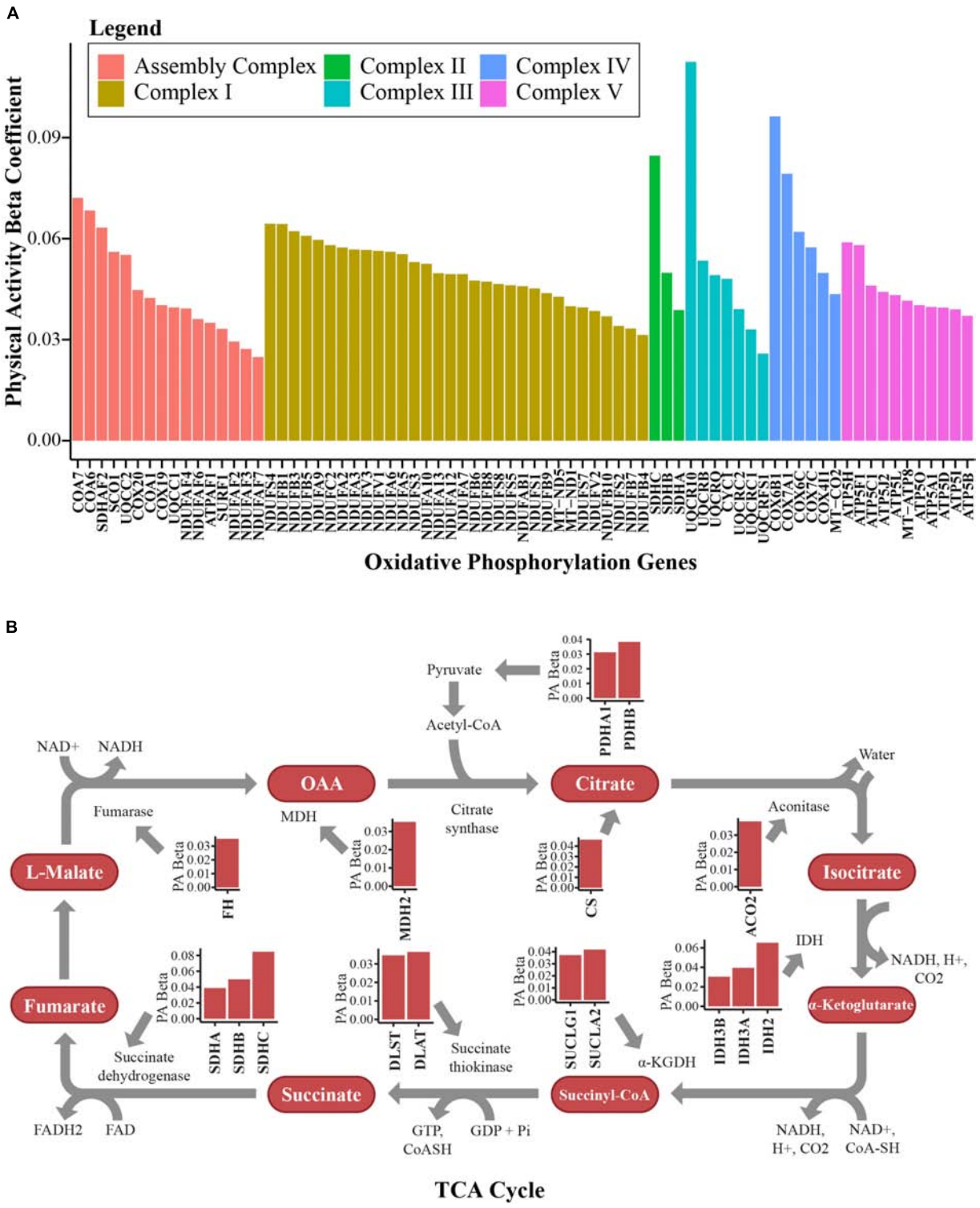

FIGURE 3 | Bioenergetics pathways upregulated with higher physical activity in human skeletal muscle. **(A)** Proteins associated with oxidative phosphorylation. Proteins from complex I, complex II, complex III, complex IV, and complex V in the electron transport chain and assembly complex proteins were overrepresented with higher physical activity. The *y*-axis shows the log2 abundance change of the protein associated with one higher point in the physical activity score (0–3) and the *x*-axis shows the gene name associated with each protein. Different complexes are color coded. **(B)** Many TCA cycle proteins were overrepresented with higher physical activity. All significantly quantified proteins are shown as bar plots. Level of significance of each protein was assessed by lmerTest from the mixed model regression results and the protein was reported as significant if the $p < 0.05$.

regulators of antigen processing endoplasmic reticulum aminopeptidase 2 (ERAP2) (de Castro and Stratikos, 2018), and T-cell activation drebrin-like protein (DBNL) (Le Bras et al., 2004). Other proteins that modulate NFκB signaling were also downregulated, including ubiquitin conjugating enzyme E2 V1 (UBE2V1), which activates NFκB (Syed et al., 2006; Flowers et al., 2018), and OTU deubiquitinase 7B (OTUD7B), a clock-regulated protein that downregulates the non-canonical NF-κB pathway (Hou et al., 2017; 48: 939–950). Lower levels of immunoglobulin heavy constant alpha 2 (IGHA2) and immunoglobulin kappa constant (IGKC) in muscle of individuals with higher physical activity indicated reduced recruitment of B lymphocytes (**Table 2**). Unsurprisingly, unlike all other immune proteins, NLR family member X1 (NLRX1), a protein that regulates mitochondrial antivirus response that is located on the outer mitochondrial membrane, was positively associated with physical activity (Jaworska et al., 2014).

High intensity training may cause mechanical damage to skeletal muscle, followed by an inflammatory response and increased levels of proteins connected with apoptosis and necrosis (Podhorska-Okolow et al., 1998). However, this was not observed with participants in this study, even those who were engaged in high intensity training, as proteins responsible for inflammatory response and apoptosis were downregulated, likely due to relatively little acute damage. For example, higher physical activity was associated with decreased expression of WD repeat domain 92 (WDR92, beta = −0.027, p = 0.0485), B cell receptor associated protein 31 (BCAP31, beta = −0.029, p = 0.001), and phosducin-like 3 (PDCL3, beta = −0.037, p = 0.00008) – proteins which exert pro-apoptotic activity by interacting with different caspases. Similarly, B-cell lymphoma 2 protein – a strong pro-apoptotic protein that opens a mitochondrial voltage-dependent anion channel that causes loss of membrane potential and cytochrome *c* release – was also downregulated. However, proteins described as anti-apoptotic, such as myeloid derived growth factor (MYDGF, beta = −0.047, p = 0.006) and major facilitator superfamily domain containing 10 (MFSD10, beta = −0.089, p = 0.010), were also lower

TABLE 2 | Immune related proteins and physical activity.

Gene name	Uniprot ID	Location	Protein description	Physical activity beta	Physical activity *p*-value
ERAP2	ERAP2_HUMAN	Endoplasmic reticulum	Endoplasmic reticulum aminopeptidase 2	−0.1486	0.0002
IGHA2	IGHA2_HUMAN	Cell membrane	Immunoglobulin heavy constant alpha 2	−0.1291	0.0184
IGKC	IGKC_HUMAN	Cell membrane	Immunoglobulin kappa constant chain C region TI	−0.1063	0.0360
C1RL	C1RL_HUMAN	Other	Complement C1r subcomponent-like protein	−0.0661	0.0454
CAPG	CAPG_HUMAN	Nucleus	Macrophage-capping protein	−0.0594	0.0422
CFD	CFAD_HUMAN	Other	Complement factor D	−0.0591	0.0184
WDFY1	WDFY1_HUMAN	Nucleus	WD repeat and FYVE domain-containing protein 1	−0.0397	0.0135
OTUD7B	OTU7B_HUMAN	Nucleus	OTU domain-containing protein 7B	−0.0350	0.0033
CHID1	CHID1_HUMAN	Lysosome	Chitinase domain-containing protein 1	−0.0329	0.0064
DBNL	DBNL_HUMAN	Cell membrane	Drebrin-like protein	−0.0324	0.0378
UBE2V1	UB2V1_HUMAN	Nucleus	Ubiquitin-conjugating enzyme E2 variant 1	−0.0311	0.0148
OTUB1	OTUB1_HUMAN	Nucleus	Ubiquitin thioesterase OTUB1	−0.0294	0.0007
MYO18A	MY18A_HUMAN	Endoplasmic reticulum	Unconventional myosin-XVIIIa	−0.0285	0.0083
INPPL1	SHIP2_HUMAN	Membrane	Phosphatidylinositol 3,4,5-trisphosphate 5-phosphatase 2	−0.0206	0.0042
ABCC9	ABCC9_HUMAN	Membrane	ATP-binding cassette sub-family C member 9	−0.0202	0.0470
EIF2AK2	E2AK2_HUMAN	Nucleus	Interferon-induced, double-stranded RNA-activated protein kinase	−0.0188	0.0115
PCBP2	PCBP2_HUMAN	Nucleus	Poly(rC)-binding protein 2	−0.0148	0.0297
NLRX1	NLRX1_HUMAN	Mitochondrion outer membrane	NLR family member X1	0.0183	0.0343
C1QBP	C1QBP_HUMAN	Mitochondrion matrix	Complement component 1 Q subcomponent-binding protein	0.0362	0.0066

Immune related proteins were significantly underrepresented in skeletal muscle of participants who reported higher levels of physical activity. Level of significance of each protein was assessed by lmerTest from the mixed model regression results and the protein was reported as significant if the $p < 0.05$. Of note, all immune proteins were negatively correlated with physical activity (negative beta), except for NLRX1, and C1QBP.

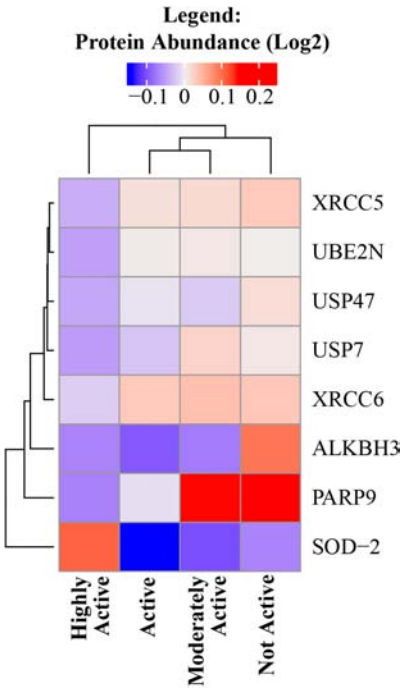

FIGURE 4 | DNA damage and DNA repair proteins decreased in abundance with higher physical activity. All significant DNA damage and DNA repair proteins were negatively correlated with physical activity except SOD-2. The level of significance of each DNA damage protein was assessed by lmerTest from the mixed model regression results and the protein was reported as significant if the $p < 0.05$. Heat map shows four physical activity levels (x axis) and the average abundance of significant DNA damage and DNA repair proteins correlated with physical activity levels (y axis). Dark blue suggests lower relative log2 abundance of the protein and red suggests higher log2 relative protein abundance. The range of log2 abundance of the protein is from –0.1 to 0.2 (legend on top). The similarity between genes and physical activity groups are represented by hierarchical clustering on y- and x-axis, respectively.

with higher physical activity, consistent with the apoptosis mechanism being balanced in physically active individuals (Phaneuf and Leeuwenburgh, 2001).

Exercise can cause an acute increase in energetic metabolism that unavoidably results in greater production of reactive oxygen species (ROS) by mitochondria, which can cause oxidative damage to DNA, proteins, and other macromolecules. However, as an individual continues to exercise, multiple antioxidant mechanisms are upregulated to buffer the damaging effects of ROS (Gomez-Cabrera et al., 2008; Hitomi et al., 2008; He et al., 2016). Indeed, superoxide dismutase (SOD) acts as the first line of defense in skeletal muscle against oxidative stress and SOD-2 is significantly overrepresented with higher physical activity (beta = 0.04, p = 0.005). In accordance with this view, a number of DNA damage and DNA repair proteins decreased in abundance with higher physical activity – such as poly(ADP-ribose) polymerase family member 9, alkylated DNA repair protein AlkB homolog 3, X-ray repair cross-complementing 5 and 6 (Lu et al., 2018), ubiquitin conjugating enzyme E2 N, and replisome-enriched small ubiquitin-like modifiers deubiquitinase – which are essential for DNA replication (**Figure 4**).

Senescent cells accumulate progressively with aging in tissues and organs (He and Sharpless, 2017). Current research is focused on determining if terminally differentiated, non-replicating cells – such as myocytes – may develop a specific form of senescence, while the impact of age and physical activity on senescence phenotypes in muscle is also under investigation. In this study, several proteins that are annotated as senescence-associated were underrepresented in participants who reported higher levels of physical activity (e.g., MAP2K3 and EIF3A), with the exception of mitochondria-localized senescent proteins, which were instead overrepresented (**Supplementary Table S4**). The role of these proteins in the changes that occur in intermyocellular adipocytes, infiltrated immune cells, or myocytes – or rather in satellite cells, which is unlikely given the small volume of these cells – is unclear and should be addressed by future studies.

Physical Activity Affects the Spliceosome

The spliceosome is organized into 6 different well-recognized protein complexes (Matera and Wang, 2014) that process pre-mRNA transcripts and cause different combinations of exons (and sometime introns) to produce a variety of splicing protein variants from the same gene. Alternative splicing in skeletal muscle regulates myogenesis, muscle contraction, and calcium handling in myofibers. There are ∼70 genes that undergo both transcriptional and splicing regulation during myogenesis (Trapnell et al., 2010) and 95 alternative splicing events that undergo robust and conserved splicing changes during the course of myogenic differentiation (Bland et al., 2010). The number of genes that are differentially spliced and the number of splicing errors that produce non-functional proteins tend to increase with age (Rodriguez et al., 2016; Saudemont et al., 2017). Previous studies have proposed that changes in alternative splicing with aging may be reflective of an adaptive strategy aimed at compensating for the accumulation of molecular damage (Latorre and Harries, 2017). In this study, ∼99 spliceosome proteins were quantified, of which 24 were significantly downregulated with physical activity (**Figure 5**). Speculatively, this may indicate that the requirements for compensation are reduced due to less accumulated damage. However, there is limited evidence connecting abundance of splicing machinery proteins and changes in the number and type of splicing variants (such as progerin) produced during transcription and translation related to senescence (Deschenes and Chabot, 2017). Interestingly, the abundance of the aging-related laminin-A splice variant progerin (beta = −0.023, p = 0.0063) was lower in highly active individuals along with other spliceosome proteins. Although a causal relationship between downregulation of splicing proteins and lower progerin levels (in addition to other splicing variants) cannot be established from our observational data, this hypothesis should be addressed in future studies. Nevertheless, the data from this study suggest that changes in the splicing machinery that occur with aging might

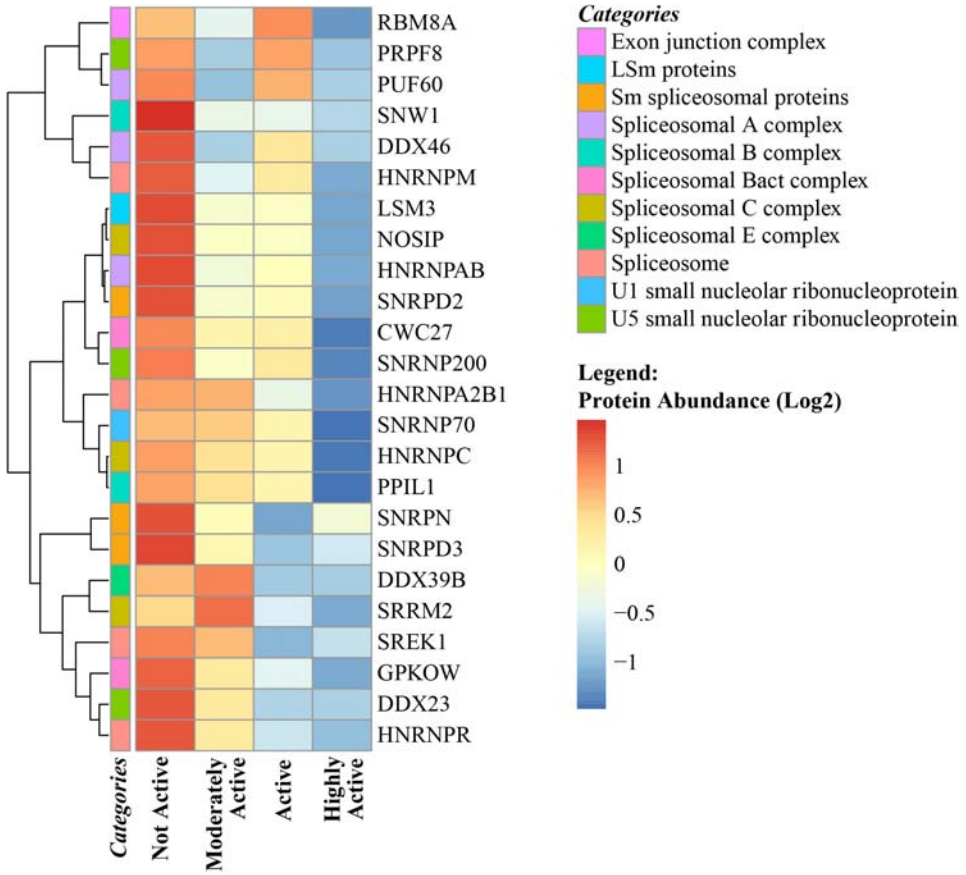

FIGURE 5 | Spliceosome complex proteins in skeletal muscle and physical activity. All significant spliceosome complex proteins were negatively correlated with physical activity. The level of significance of each spliceosome protein was assessed by lmerTest from the mixed model regression results and the protein was reported as significant if the $p < 0.05$. The heat map correlates four physical activity levels (*x*-axis) with the average abundance of spliceosomes genes correlated with physical activity levels (*y*-axis). Dark blue suggests negative correlation and red suggests positive correlation. The normalized range of the protein abundance for physical activity correlation is shown from red to blue (legend on bottom right). The color-coded "categories" column of the heatmap shows the spliceosome complex category annotated for each gene and the spliceosome category annotation label legend on top right shows the spliceosome major complex categories. Similarity between the spliceosome genes are shown by hierarchical clustering and represented with the dendrogram on the left *y*-axis.

be offset by regular physical activity, although the relevance to human health of this effect remains to be established.

Physical Activity Affects Autophagy and Proteostasis

Muscle contraction during exercise increases intracellular calcium and causes mechanical and chemical stresses that trigger the unfolded protein response (Vainshtein et al., 2014). In addition, the accelerated pace of oxidative phosphorylation increases ROS production, damaging membrane lipids and proteins, while also potentially impairing organellar function, including mitochondria. This potential accumulation of damage is counteracted by the activation of macroautophagy and chaperone-mediated autophagy (Grumati et al., 2011; He et al., 2012). Contrary to previous observations, this study revealed that proteins involved in the activation/inhibition of autophagy – such as V-type proton ATPase 116 kDa subunit a isoform 1 (ATP6V0A1), apoptosis regulator BAX, biogenesis of lysosome-related organelles complex 1 subunit 1 (BLOC1S1), heat shock protein HSP 90-alpha (HSP90AA1), Ras-related protein Rab-7a (RAB7A), and heat shock cognate 71 kDa protein (HSPA8) – were underrepresented in muscle biopsies from participants who were highly active. In contrast, β-glucuronidase (GUSB), an important lysosomal enzyme, and 5′-AMP-activated protein kinase subunit gamma-2 (PRKAG2), a component of AMP kinase that is the main energy-sensor that responds to changes in the cellular AMP:ATP ratio and regulates the balance between ATP production and consumption, were greater with higher physical activity, suggesting a tightly monitored balance between energy production and utilization (Mounier et al., 2015). Overall, 62 proteins related to proteostasis were quantified and were significantly associated with physical activity; 47 were underrepresented with higher physical activity, including 6 chaperonins, 20 chaperones, including 3 heat shock proteins 70, 3 heat shock proteins 90 and 1 small heat shock protein (sHSP), 7 co-chaperones, 6 DNAJ (HSP40), and 8 other proteostasis proteins (**Supplementary Table S5**). Of the 62

noted proteostasis proteins, 15 proteins were overrepresented in the muscle from participants who reported higher levels of physical activity, including 7 chaperones (including an HSP70), 3 DNAJ (HSP40) proteins, and 5 other proteostasis proteins. DNAJB2 transcript has been previously reported to be upregulated in human skeletal muscle during recovery from exercise-induced damage (Mahoney et al., 2008). sHSPs play an important role in cytoskeleton protection and a bout of eccentric exercise induces rapid accumulation of sHSPs (HSPB1 and HSPB5) and later accumulation of HSP70 (Paulsen et al., 2007). Our study revealed that some specific chaperones and sHSPs were underrepresented, while others were overrepresented based on physical activity, which could due to differences between selective cell structures when reacting to regular physical activity that induces significant levels of damage accumulation. Further investigation is required to clarify this discrepancy.

Summary of Conclusion

In this study, we used discovery proteomics to understand how daily physical activity may affect skeletal muscle biology. This approach is substantially different from previous proteomic studies that have characterized the effect of acute increased physical activity or intense training, which can be conceptualized as a dynamic response to stress that is aimed at reaching a different level of equilibrium (Margaritelis et al., 2018). In contrast, this study examined individuals who had not dramatically changed their level of physical activity over the last few months, in order to understand what adaptive changes are maintained in active muscle over a long period. It must be noted that, although study participants were 20–87 years of age, they were all extremely healthy according to very strict and objective inclusion criteria and all individuals were not medicated. The most evident result, namely that physical activity was associated with higher structural and functional mitochondrial proteins, was not unexpected. However, it is important to note that none of the participants had performed an acute bout of intense physical activity directly before the muscle biopsy and, therefore, these findings demonstrate that maintaining a good level of regular physical activity may be sufficient to counteract the declining mitochondrial health that occurs in many aging individuals, which can result in negative metabolic and functional consequences (Choi et al., 2016; Zane et al., 2017). Interestingly, the expanded energy machinery was not associated with increased DNA damage due to oxidative stress, as evidenced by unchanged levels in DNA damage/repair proteins. Consistent with previous studies, there was evidence of enhanced antioxidant mechanisms, including overexpression of SOD and SOD-2 (de Sousa et al., 2017; Bouzid et al., 2018).

Interestingly, immune-related proteins were underrepresented in the muscle of participants who were physically active, including proteins related to both innate and adaptive immunity, which is also consistent with the literature and opposes the proinflammatory state that is typical of aging (de Sousa et al., 2017). For example, Duggal et al. examined the immune profiles of 125 middle-aged and older adults who had been active cyclers for much of their adult lives, comparing them to 75 age−matched sedentary controls, revealing that physical activity protects against many aspects of immunosenescence, including thymic involution, and circulating levels of inflammatory markers (Duggal et al., 2018).This is the first study to demonstrate that usual physical activity in daily life is associated with lower inflammation in the muscle of healthy individuals. Inflammation is associated with satellite cell dysfunction and impaired muscle regeneration and, thus, increased inflammation may be one of the mechanisms that leads to sarcopenia with aging (Perandini et al., 2018). Indeed, interventions aimed at reducing inflammation have been proposed for preventing age-associated sarcopenia, although currently there is no solid evidence regarding efficacy (Alturki et al., 2018).

Unexpectedly, this study revealed a strong association between higher physical activity and lower expression of spliceosome complex proteins. Previous studies have demonstrated that expression of dysregulated splicing factor is associated with aging in humans, although the direction of this association remains controversial (Lee et al., 2016). However, this is the first study to show that physical activity is associated with a lower representation of spliceosome in skeletal muscle. The rationale for changes in spliceosome and how these changes differ from those that occur in normal aging are unknown. Alternative splicing is one mechanism used by mammals to counteract the damage accumulation that occurs with stress and aging (Li et al., 2017). Thus, physical activity may prevent damage accumulation in muscle and simultaneously prevents the activation of this potential mechanism of resilience. In this study, changes in splicing machinery proteins were complemented with data on qualitative or quantitative differential representation of splicing variants and this is an important direction for future research.

This study has unique strengths that serve as an important addition to the literature. First, this study utilized a relatively large sample of individuals who were extremely healthy based on strict, highly standardized criteria. Further, we used a quantitative proteomics approach using isobaric tags, which allowed for the quantification of a large set of proteins. The findings described and discussed herein are robust because they are not based on changes in one single protein, but instead are based on detecting harmonic changes in a large set of proteins that cluster in the same pathways. However, this study does have limitations, especially in terms of population sample, and will need to be replicated in an independent sample before the findings can be generalized. Also, although we used strict, highly standardized criteria to define healthy status in our study population, there is no guarantee that younger people who meet such criteria will remain healthy through mid- and late-life, and therefore, the results from this study need to be confirmed longitudinally. Finally, this study was conducted using a very healthy population in order to increase homogeneity of the findings and to avoid potential confounding effects of disease and treatments. Thus, our findings may not apply to persons who are affected by disease or are chronically medicated.

In conclusion, our study provides evidence that high levels of physical activity counteract some of the effects of aging, namely the decline in mitochondrial volume (and function) and in pro-inflammatory status. We also revealed a robust change in the splicing machinery, the functional meaning of which should be addressed in future studies.

DATA AVAILABILITY

The datasets generated for this study can be found in PRIDE, dataset identifier PXD011967.

AUTHOR CONTRIBUTIONS

LF, RS, ES, and CU-M designed the study and performed the analyses. LF, CC, and MG-F performed and collected the muscle biopsies. AL, CU-M, and RM generated the data. All authors analyzed the data, wrote the manuscript, and gave final approval for publication.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphys.2019.00312/full#supplementary-material

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Influence of Habitual Physical Behavior – Sleeping, Sedentarism, Physical Activity – On Bone Health in Community-Dwelling Older People

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Sedentary behavior (SB) has emerged as an independent public-health risk and may contribute to the lower bone mineral density (BMD) in old (>60 years of age) than young adults. The purpose of this study was to quantify SB and habitual physical behavior (PB) in community-dwelling older adults and how this correlates with BMD. In 112 relatively healthy and independent-living individuals aged 72.5 ± 6.4 years, BMD, PB and SB were determined using dual energy X-ray absorptiometry and 7-day three-dimensional accelerometry, respectively. In men, only healthy and osteopenic BMDs were found, whereas in women, osteoporotic BMD classifications also occurred. Our sample spent ∼61%, 7%, 12% and 19% of daily waking hours in SB, standing, LIPA [light intensity physical activity (PA)] and MVPA (medium-to-vigorous intensity PA), respectively. In men, after accounting for covariates (BMI, total fat, android:gynoid ratio), sleeping (hours/day), number of breaks in SB, number of SB ≥ 5 min, number of PA bouts, total duration of PA bouts (min), mean PA bouts duration (min), LIPA (%PA bout time) and MVPA (%PA bout time) were all predictors of BMD. In women, after accounting for covariates (age, BMI, total fat, android:gynoid ratio), SB (hours/day), SB (% waking hours), LIPA (hours/day), LIPA (% waking hours), MVPA (% waking hours) and number of short SB (i.e., <5 min), total time spent in PA (min) significantly correlated with BMD. In conclusion, the PB predictors of bone health in older persons include: night time sleeping duration, number of short bouts of SB, number and duration of bouts of PA relative to total waking hours. While radar graphs of PB patterns for healthy, osteopenic, osteoporotic individuals highlighted significant differences in PB between them, they were not consistent with the expectations from the Mechanostat Theory: i.e., more loading leads to better bone. Rather, our results suggest that a balance of activities must be maintained across the PB spectrum, where certain PB parameters are especially impactful in each sex, supporting the recently coined multifactorial-based variations in the Mechanostat threshold.

Keywords: accelerometry, aging, bone mineral density, physical behavior, Z-score

INTRODUCTION

In Western societies the average older-adult is highly sedentary and spends up to 80% of their waking time in sedentary behavior (SB) that increases the risk of cardio-metabolic, vascular and musculoskeletal dysfunction (Bey and Hamilton, 2003; Matthews et al., 2008; Proper et al., 2011; Wullems et al., 2016; Ryan et al., 2018a,c). During aging people suffer from a loss of bone mass (Alffram and Bauer, 1962). More than three-million individuals in the United Kingdom suffer from osteoporosis, with €1.2 billion being spent to attend to 200,000 annual fractures sustained to the hip, wrist and spine, and for preventative treatment that only maintains rather than cures this condition (NHS, 2016).

Exercise that includes activities of daily living, such as walking, has been found to be associated with a 30% lower risk of falling and fractures in elderly Caucasian women (>65 years) (Cummings et al., 1995). The reduced fall and fracture risk may not only be attributable to larger muscles, greater strength and better balance, but also to increased bone strength. The Mechanostat theory suggests that bone strength increases in response to increased strain on the bone (Frost, 1987a,b) and walking-associated mechanical loading of the skeleton by transmission of ground-reaction forces (GRFs) through locomotor muscles and respective joints and bones has been suggested to contribute to the reduced fracture risk (Kohrt et al., 1997).

However, recent research suggests that such benefits yielded from regular exercise and physical activity (PA) can be reversed equally or in greater magnitude if individuals are sedentary for the rest of their waking day following engagement in PA. For instance, adults performing ≥150 min/week of moderate to vigorous physical activity (MVPA) often display a detrimental dose–response association with total TV viewing time, typically reversing the effects of PA (Healy et al., 2008). This relationship was also recognized by others (Owen et al., 2010) who stated that adults should adhere to public-health guidelines for PA, but if they sit for prolonged periods of time, no changes to metabolic disease risk-factor profile can be witnessed. Furthermore, if prolonged SB, such as sitting, impairs metabolic functioning, it may be that age-associated bone loss is partially attributable to this lifestyle behavior.

Studies into the effects of SB and bone health have attempted to answer this with the majority of insight and understanding being provided through examining the physiological effects of prolonged bed-rest or studies incorporating zero gravity environments into their experimental design. Reduced gravitational loading of the skeleton is a common characteristic shared amongst all these procedures and contributes to both muscular atrophy and bone loss, in some instances a loss of up to 1% of trabecular bone per week (Mazess and Whedon, 1983; Whedon, 1984). Previous research in healthy young adults showed that the decrease in BMD during bed rest was attenuated with regular lower-limb loading exercise (Zerwekh et al., 1998; Kim et al., 2003). However, any association between less extreme SB (such as simply habitual sitting) and therefore more realistic for everyday persons, and bone health is scarcely studied in the young and even less so in older-age.

Given that unloading leads to a reduction in BMD, one might expect that there is a negative correlation between sleep duration and bone strength, as has indeed been observed (Lucassen et al., 2017). This is, however, equivocal and others have found no relationship between sleep duration and BMD (Niu et al., 2015) or even observed that a short sleep duration was associated with a lower BMD (Fu et al., 2011; Lin et al., 2019). It therefore follows that one of our objectives was to assess the impact of sleep on BMD.

Similarly, it is clear that sex plays a key role in differential bone quality of men compared to women, be they younger or older. In fact it is clear that even where age and genetics background is controlled for (through a study on opposite sex-twins across a large age range), the differences are such that males exhibit up to 21% greater BMD than their female counterparts at most tested sites (Naganathan and Sambrook, 2003; Nieves et al., 2005). Sex must therefore be taken into account in assessing any impact of lifestyle factors on bone health.

The purpose of the present cross-sectional study therefore, was to objectively quantify habitual physical behavior (PB) (i.e., total SB and PA, as well as patterns) using three-dimensional accelerometry to establish associations with bone health. It was hypothesized that (1) individuals engaging more frequently in light-intensity and moderate-vigorous exercise demonstrate better bone health than their less active counterparts; (2) greater SB times is associated with poorer bone health; (3) individuals breaking prolonged bouts of SB more frequently display better bone health. PB was measured over 7 days, using a 3-D accelerometer. Dual-energy X-ray absorptiometry (DEXA) was used to quantify the BMD and soft-tissue.

MATERIALS AND METHODS

Study Sample and Ethics

Participants were recruited by word-of-mouth from a number of national organizations and local clubs in Cheshire, United Kingdom [including the University of the Third Age (U3A), Rotary, Age United Kingdom, local golf clubs]. One hundred and twelve adults (men, N = 51/women, N = 61) volunteered to participate in this study. All were aged between 57 and 89 years (average ± SD = males 73.6 ± 6.2 years, females 71.6 ± 6.4 years). Participants were excluded if they were: <55 years of age, had any untreated cardiovascular disease (CVD), medication, were diabetic, had any clinically/medication-induced mobility and/or lower limbs strength limitations, had recent (<3 months) injury or surgery, presented decreased mental competence to provide informed consent or understand the study instructions. In addition, data on falls risk [Falls Risk Assessment Tool/FRAT (NICE, 2013)] served as a measure of frailty and those scoring ≥3 (i.e., high fall risk) were excluded. It is also notable that 40% of the sample utilized a primary CVD medication (e.g., statins or warfarin).

Full ethical approval was received through the Manchester Metropolitan University Ethics Committee prior to

experimentation. The investigators had completed Ionising Radiation Medical Exposure Regulations (IRMER) training (for studies involving radiation to human participants), and further in-house Risk Assessments were performed that are over and above those stipulated by IRMER, and include daily calibrations, wearing a dosimeter for the regular operator, two room dosimeters in the scanning suite, and logging of radiation dose of each scan. Informed written consent was obtained from each participant.

Anthropometrics and Body Mass Index (BMI)

Participants' height and weight were assessed (SECA Beam balance scale, Germany; Woodway PPS 70med Klima, Germany) on arrival at the laboratory in a 10-h overnight fasted state, with the participant unshod and wearing a hospital gown. Dual energy X-ray absorptiometry (Hologic, DEXA Discovery W, Reading, United Kingdom) immediately followed anthropometry and health questionnaire completion, to assess body composition and BMI calculation. Thereto, participants laid supine with palms down, fingers splayed and feet inverted to expose the fibular bone for the 7-min scanning procedure (whole body procedure, EF 8.4 lSv).

Bone Mineral Density (BMD)

The DXA scan was also used to determine bone phenotype (BMD and content), using OnePass technology to eliminate beam overlap errors and image distortion.

Data was later analyzed using Hologic APEX software (version 3.3) with each region of interest (ROI) carefully demarcated by the same researcher (with an ICC of 0.987). We selected five body segments: spine (average of lumbar and thoracic), pelvis, upper limbs (average of left and right sides), ribs (average of left and right sides) and lower limbs (average of left and right sides). Total bone BMD is also reported.

The T-scores for whole body BMD was used to classify participants as: (1) Normal, T-score < 1.0 SD below normal; (2) Osteopenic, $1 <$ T-score < 2.5 SD below or (3) Osteoporotic T-score > 2.5 SD below normal (WHO, 1994). These correspond to approximately 10–12%, 20%, and 25% lower values than that in the young healthy 30-year-old, respectively. The T-score indicates how many standard deviations of a sex-matched reference population the BMD of a participant differs from that reference population (here an average healthy 30-year-old man or woman).

Z-scores (i.e., a marker of how bone density compares against the average bone density of an age- and sex-matched group) were calculated using sex and ethnic group specific data from the national health and nutrition examination database (NHANES III).

Physical Activity (PA) and Sedentary Behavior (SB) Analysis

Sedentary behavior and PA data were collected using commercially available accelerometer hardware and software (GeneActiv Action, Activinsights Ltd., Kimbolton, United Kingdom). Accelerometry outputs were further processed using an in-house developed and validated algorithm based on age-specific activity output cut-off points (Wullems et al., 2017).

Participants were fitted with the accelerometer on their first laboratory visit using two waterproof adhesive patches (3M Tegaderm Transparent Film, Bracknell, United Kingdom). Accelerometers were worn at 50% femur length for 6–7 days post DXA scan. During these 6–7 days participants were asked to carry on with their habitual activities of daily living, exercise and resting habits including bathing, showering and swimming. They were provided with two spare adhesive patches to be applied by themselves on top of the original fittings should the adhesion start to loosen during the course of 6–7 days of monitoring. Overall 58/61 women and 51/51 men returned a complete set of PB data.

Physical behavior was then quantified by normalizing the total wear time during waking hours. SB was classified as sitting or lying or activities incurring a metabolic cost of <1.5 METs (Tremblay et al., 2010). Differing levels of PB were classified as follows:

(1) Sleeping
(2) Quiet standing
(3) Light intensity physical activity (LIPA), whereby activities incurred a metabolic cost of <3 METs.
(4) Moderate-vigorous physical activity (MVPA), whereby activities incurred a metabolic cost of >3 METs (Sasaki et al., 2016).

We have previously cross validated this accelerometer data processing approach using directly measured activities in older persons (Wullems et al., 2017). Here, we quantified 26 common components of PB [for detailed definitions read (Ryan et al., 2018a,b)]:

(1) Nine general PBs: Sleeping (hours/24 h), SB (hours/24 h), standing (hours/24 h), LIPA (hours/24 h), MVPA (hours/24 h), SB (% of waking hours), standing (% of waking hours), LIPA (% of waking hours), MVPA (in % of waking hours).
(2) Six PBs specific to SB amount and accumulation pattern: Breaks in SB (a count), <5 min SB bout (a count), ≥ 5 min SB bout (a count), mean SB bout length (min), Alfa, W50% (min).
(3) Eleven PBs specific to amount of PA and accumulation pattern: PA bouts (a count), PA bouts (total min), mean PA bout length (min), SB (%PA bout time), standing (%PA bout time), LIPA (%PA bout time), MVPA (%PA bout time), ≥ 10 min MVPA bouts (in total min), ≥ 10 min MVPA (a count), Sporadic MVPA (in min), total week ≥ 10 min MVPA (in min).

Sleeping was defined as the overnight period in bed. The time of going to bed and getting out of bed was noted down in a diary by the participants and verified by absence of accelerations in the z-direction during this period.

Statistical Analyses

All analyses were performed using SPSS Version 24 (IBM, Chicago, IL, United States) whereby all data

was checked for Parametricity, with tests of normality (Kolmogorov–Smirnov) being conducted each for men and for women separately.

Associations

Bivariate correlations (in men only and women only) were then conducted to assess the influence that PA and SB parameters held over total and site-specific BMD. Results following bivariate correlations are displayed as correlation coefficient (r). Partial correlation controlling for known covariates (see below) that influence BMD then took place with coefficients reported as $R_{adj}{}^{n}$ depending on previously established bivariate significant associations.

Covariates

Particular risk factors for low BMD were identified from previous research including age (Riggs et al., 1982; Manolagas and Jilka, 1995), total fat mass (Harris et al., 1992), and general anthropometry. These data were therefore also collected at study onset. The impact of these potential covariates in our study sample was assessed using Pearson (or Spearman' Rho for non-normal data) bivariate correlation against bone parameters.

Z_{PB}-Score Graphic Representation of Group Physical Behavior Patterns

For the graphical representation (Microsoft Excel, Version 2013, Washington, DC, United States) of participants' habitual PB categorized by their bone health (Z-scores sub-populations), we utilized radar graphs as these are arguably a more comprehensive way to contrast the overall PB of healthy vs. unhealthy bone phenotypes. To compare between the PB of the grouping variables (normal range vs. osteopenia vs. osteoporosis) we used ANOVA (three levels of BMD: <0.75 vs. <0.9 vs. $\geq$0.9 g/cm^2) or Kruskal–Wallis tests as appropriate, with follow-up *post hoc* pairwise comparisons (Bonferroni corrected unpaired t-tests for the former, or Mann–Whitney tests for the latter) where necessary. Here, PB parameters (total amounts and patterns) were standardized to unit-less quantities into Z-scores to obtain an overall PB picture (whereby PB_Z-score = [mean of group – mean of sample] ÷ standard deviation of sample). Unit weighed score (to compute radar graph areas) assigned a negative sign to PBs linked to unloading bones and a positive sign to those linked to loading of bones sites. The quantitative calculation of the differences between the groups' areas in the radar graph, (between PB Z_{PB}-scores of the bone health groupings) was conducted by computing the Z_{PB}-*scores* distance using the NORMDIST function in Microsoft EXCEL. This function returns the distance as a percentage of area.

For all inferential tests, statistical significance was accepted at $\alpha \leq 0.05$. In this sample of 61 women, threshold for a $\beta = 0.80$ in the correlations, required an explained variance of $r^2 = 0.12$ (i.e., $r = 0.346$). In this sample of 51 men, threshold for a $\beta = 0.80$ in the correlations, required an explained variance of $r^2 = 0.14$ (i.e., $r = 0.374$).

RESULTS

Demographics and BMD

Based on their answers to the health questionnaires during the DEXA scanning procedure, it was ascertained that: 88 participants scored low risk in the FRAT, 87 had no history of major illness, 71 were currently using statins, 102 were non-smokers, 81 had not carried out any resistance exercise in the 6 months preceding the laboratory assessments, 5 regularly consumed dairy products, 18 seldom consumed caffeinated products, 101 did not have rheumatoid arthritis, 98 consumed less than three units of alcohol per day and finally 90 took no calcium/vitamin D supplements.

Participants age, anthropometry and bone health (BMD at five sites and total BMD and Z-score) are detailed in **Table 1** separately for men and women. The two sexes were well matched for age and BMI.

Men Only Sample

In men (total n = 51), BMD of the ribs was marginally below the 'healthy' classification, suggestive of osteopenia at this site (average left and right ribs BMD: 0.81 ± 0.10g/cm^2). Notably, however, BMD in all other regions and especially the weight-bearing regions tended to be well within the healthy range. The overall average Z-score in the men varied greatly (Z-score: 1.57 ± 1.68).

Women Only Sample

Women (total n = 61). Worryingly the average BMD of the ribs was suggestive of osteoporosis (BMD < 0.75g/cm^2). The rest of the BMD sites inferred healthy bone (BMD > 0.9g/cm^2). The overall average Z-score in the females (Z-score: 0.89 ± 1.11) was greatly varied across the 'healthy to osteopenic spectrum' against age and sex matched population, thereby suggesting there would be factors modulating where any individual older woman's bone health score would reside.

TABLE 1 | Study population anthropometry and bone characteristics by sex.

	Men	Women
Age (years)	**73.6 ± 6.2 ND**	**71.6 ± 6.4ND**
Mass (kg)	79.1 ± 11.8ND	67.3 ± 13.1ND
Height (cm)	173.4 ± 7.6	160.2 ± 5.6ND
BMI (kg/m^2)	26.3 ± 3.9	**26.2 ± 5.0ND**
Total fat (kg)	24.3 ± 6.7ND	28.3 ± 8.9ND
Android:gynoid ratio	0.49 ± 0.12ND	0.37 ± 0.11ND
Ribs	0.81 ± 0.10ND	0.65 ± 0.00ND
Spine	1.23 ± 0.24ND	0.97 ± 0.02ND
Pelvis	1.28 ± 0.22	1.15 ± 0.22
Upper_limbs	1.79 ± 0.16ND	1.42 ± 0.19
Lower_limbs	2.61 ± 0.34	2.09 ± 0.28
Total	**1.32 ± 0.20**	**1.09 ± 0.12ND**
Z_score	**1.57 ± 1.68**	**0.89 ± 1.11**

ND denotes a normally distributed data set. Data are presented as mean ± SD. Bold numbers indicate significant correlations.

TABLE 2 | Bivariate correlation analysis of age, BMI, and fat as potential covariates for BMD.

(A) Correlation coefficients in

Men	Age	BMI	Total fat	A:G ratio
Ribs	0.124	0.182	0.000	0.160
Spine	0.124	**0.278***	0.027	0.083
Pelvis	−0.097	**0.293***	0.215	0.128
Upper_limbs	−0.023	**0.290***	0.103	**0.379****
Lower_limbs	0.024	**0.322***	**0.325***	0.058
Total	0.042	0.163	0.121	0.065
Z_score	0.021	0.198	0.179	0.073

(B) Correlation coefficients in women

Women	Age	BMI	Total fat	A:G ratio
Ribs	−0.087	**0.382****	**0.332****	−0.063
Spine	0.030	**0.445****	**0.368****	0.039
Pelvis	−0.165	**0.473****	**0.433****	**0.216***
Upper_limbs	**−0.241***	**0.384****	**0.401****	0.125
Lower_limbs	−0.061	**0.571****	**0.511****	**0.237***
Total	0.132	**0.399****	**0.344****	0.139
Z_score	**0.221***	**0.350****	**0.237***	0.170

*Analysis was performed between each presumed covariate and specific bone BMD site by sex *P < 0.05; **P < 0.01; correlation coefficients are presented. Plain for Pearson and in shaded gray boxes for Spearman' Rho.* ***(A)*** *Correlation coefficients in men.* ***(B)*** *Correlation coefficients in women.*

Covariates

The covariates analyses against BMD sites can be seen in **Tables 2A,B**. Significant covariates were confirmed in men thus: BMI for spine, pelvis, upper limbs and lower limbs; total fat mass for the lower limbs; android:gynoid ratio for the upper limbs. In women, covariates included: age for upper limbs and total Z-score; both BMI and fat mass for all sites (i.e., ribs, spine, pelvis, upper limbs, lower limbs, total BMD, Z-score); android:gynoid ratio for pelvis and lower limbs. It was noteworthy in this limited age range (females average of 71.6 ± 6.4 years and men average of 73.6 ± 6.2 years), that age was seldom a covariate either site specific BMD and/or overall Z-score in women and not at all for men

Physical Behavior in Men and Women

Participants' habitual physical behaviors or PB (9 general markers, 6 markers related specifically to SB accumulation and pattern, and 11 related specifically to PA accumulation and pattern) are detailed in **Table 3**.

Men Only Sample

During an average day, men spent 8.2 ± 0.7 h sleeping and their waking hours were dominated by SB (61.4 ± 8.9% waking hours), followed by MVPA (19.5 ± 5.4% waking hours) and LIPA (12.1 ± 3.8% waking hours). The average bout length of SB was 31.1 ± 9.2 min, and was longer ($p < 0.001$) than PA (standing, LIPA, MVPA combined) bout length which averaged at 16.0 ± 4.4 min.

Women Only Sample

During an average day, women slept slightly more than men with 8.5 ± 0.7 h sleeping. Like men, their waking hours were dominated by SB (61.0 ± 9.8% waking hours), followed by MVPA (18.7 ± 5.5% waking hours) and LIPA (13.2 ± 4.0% waking hours). With SB being the most prevalent behavior, it was also noted that each bout length was 31.1 ± 10.8 min. This SB bout length was longer ($p < 0.001$) than PA (standing, LIPA, MVPA combined) bout length which averaged at 16.6 ± 5.1 min.

Bivariate Correlations

Men Only Correlations

The correlations between BMD and general PB for men can be seen in **Table 4A**. Sleep time was the only behavior to show any significant association with men's BMD and this was limited to negative associations with the BMD of the pelvis ($R = -0.307$, $p < 0.05$), upper limbs ($R = -0.374$, $p < 0.01$) and whole body Z-score ($R = -0.261$, $p < 0.05$) indicating that more sleep was associated with lower BMD.

Looking into SB in more details in men (**Table 5A**), we found that the number of breaks in sedentarism was positively associated with upper limbs BMD ($R = 0.403$, $p < 0.01$), ribs BMD ($R = 0.282$, $p < 0.05$), total BMD ($R = 0.330$, $p < 0.01$) and total Z-score ($R = 0.296$, $p < 0.05$). Interestingly also, the number of short bouts (<5 min) in SB was positively associated with upper limbs BMD ($R = 0.238$, $p < 0.05$). Unexpectedly the number of longer bouts (>5 min) in SB was positively associated with ribs BMD ($R = 0.349$, $p < 0.01$, respectively), lower limbs ($R = 0.290$, $p < 0.05$), total BMD ($R = 0.373$, $p < 0.01$) and total Z-score ($R = 0.283$, $p < 0.05$).

As for detailed PA in men (**Table 6A**), the number of bouts of PA was positively associated with ribs BMD ($R = 0.282$, $p < 0.05$), lower limbs ($R = 0.238$, $p < 0.05$), total BMD ($R = 0.330$, $p < 0.01$) and total Z-score ($R = 0.296$, $p < 0.05$). It was interesting also to note the positive association between MVPA (as a percent of total PA time) and lower limbs BMD ($R = 0.285$, $p < 0.05$). It was surprising that the total duration of PA was only associated, and this negatively, with lower limbs BMD ($R = -0.254$, $p < 0.05$). Equally surprising was that the mean PA bout length was negatively associated with spine BMD ($R = -0.248$, $p < 0.05$), lower limbs BMD ($R = -0.396$, $p < 0.01$), total BMD ($R = -0.445$, $p < 0.01$) and the Z-score ($R = -0.362$, $p < 0.01$). Another unexpected set of results were the negative associations between LIPA and upper limbs BMD ($R = -0.289$, $p < 0.05$), lower limbs BMD ($R = -0.414$, $p < 0.01$) and total BMD ($R = -0.338$, $p < 0.05$). No other associations were significant in the men only sample.

Women Only Correlations

Women only correlations between BMD and general PB can be seen in **Table 4B**. Sleep time was in fact positively associated with whole body Z-score ($R = 0.225$, $p < 0.05$). Unexpectedly, SB (in total hours per day) was positively associated with spine BMD ($R = 0.233$, $p < 0.05$), lower limbs BMD ($R = 0.272$, $p < 0.05$) and total BMD ($R = 0.317$, $p < 0.01$). Unexpectedly also, LIPA was negatively associated with all but one site including spine BMD ($R = -0.225$, $p < 0.05$), pelvis BMD ($R = -0.242$, $p < 0.05$),

TABLE 3 | Physical behavior of the study population.

		Men	Women
General	Sleeping (hours/24 h)	8.23 ± 0.68[ND]	8.50 ± 0.67
	SB (hours/24 h)	9.68 ± 1.44[ND]	9.44 ± 1.48[ND]
	Standing (hours/24 h)	1.10 ± 0.44[ND]	1.11 ± 0.41[ND]
	LIPA (hours/24 h)	1.91 ± 0.62[ND]	2.05 ± 0.64[ND]
	MVPA (hours/24 h)	3.08 ± 0.89[ND]	2.90 ± 0.86[ND]
	SB (in %/waking hours)	61.44 ± 8.94[ND]	61.03 ± 9.84[ND]
	Standing (%/waking hours)	6.97 ± 2.77[ND]	7.14 ± 2.58[ND]
	LIPA (%/waking hours)	12.10 ± 3.82	13.15 ± 3.96[ND]
	MVPA (%/waking hours)	19.49 ± 5.38[ND]	18.68 ± 5.50[ND]
Sedentary behavior	Breaks in SB (*n*)	22.50 ± 3.80[ND]	22.02 ± 3.33[ND]
	<5 min SB bout (*n*)	6.21 ± 1.83[ND]	6.40 ± 2.14[ND]
	≥5 min SB bout (*n*)	17.06 ± 2.70[ND]	16.41 ± 1.92[ND]
	Mean SB bout length (min)	31.41 ± 9.34	31.29 ± 10.72
	Alfa	1.45 ± 0.04[ND]	1.44 ± 0.04
	W50% (min)	52.92 ± 15.11	53.65 ± 14.19[ND]
Physical activity	PA bouts (*n*)	22.50 ± 3.81[ND]	22.02 ± 3.33[ND]
	PA bouts (total min)	346.75 ± 88.01[ND]	361.75 ± 99.83[ND]
	Mean PA bout length (min)	15.75 ± 4.31[ND]	16.86 ± 5.24[ND]
	SB (%PA bout time)	1.24 ± 0.65	1.25 ± 0.73
	Standing (%PA bout time)	18.10 ± 5.30	18.93 ± 5.35[ND]
	LIPA (%PA bout time)	31.62 ± 4.91[ND]	33.63 ± 5.85[ND]
	MVPA (%PA bout time)	49.04 ± 7.78[ND]	46.20 ± 8.82[ND]
	≥10 min MVPA bouts (total min)	15.16 ± 18.40	11.01 ± 16.32
	≥10 min MVPA (*n*)	0.86 ± 0.85	0.59 ± 0.71
	Sporadic MVPA (min)	162.29 ± 42.14[ND]	166.96 ± 53.29[ND]
	Total week ≥ 10 min MVPA (min)	104.34 ± 126.34	76.45 ± 114.31

[ND] *denotes a normally distributed data set. Data are presented as mean @ SD.*

upper limbs BMD ($R = -0.230$, $p < 0.05$), lower limbs BMD ($R = -0.320$, $p < 0.01$), total BMD ($R = -0.337$, $p < 0.01$), and total Z-score ($R = -0.258$, $p < 0.05$). A similar pattern was seen with SB amount expressed as a percent of waking hours. Also unexpected were the negative associations between average daily hours spent in MVPA against spine BMD ($R = -0.256$, $p < 0.05$), total BMD ($R = -0.350$, $p < 0.01$) and total Z-score ($R = -0.224$, $p < 0.05$). A similar pattern was seen with MVPA amount expressed as a percent of waking hours (though not with the Z-score). No other correlations with general PB were significant in the women only sample.

Looking into SB in more details in women (**Table 5B**), we found that the number of breaks in sedentarism was positively associated with ribs BMD ($R = 0.266$, $p < 0.05$), pelvis BMD ($R = 0.232$, $p < 0.05$) and lower limbs BMD ($R = 0.299$, $p < 0.05$). We also found that the count of short duration (≤5 min) sedentary activities was positively associated with ribs BMD ($R = 0.328$, $p < 0.01$), upper limbs BMD ($R = 0.230$, $p < 0.05$) and lower limbs BMD ($R = 0.334$, $p < 0.01$). Finally, W50% (i.e., the bout duration below which half of all sedentary time is accrued) was negatively associated with ribs BMD ($R = -0.224$, $p < 0.05$).

As for detailed PA in women (**Table 6B**), this showed very few bivariate associations with bone health. Thus, the number of bouts of PA was positively associated with ribs BMD ($R = 0.266$, $p < 0.05$), pelvis BMD ($R = 0.232$, $p < 0.05$) and lower limbs ($R = 0.299$, $p < 0.05$). Total duration of PA was positively associated with ribs BMD ($R = 0.265$, $p < 0.05$). Mean PA bout length was negatively associated with Z-score ($R = -0.235$, $p < 0.05$). Finally the total weekly accumulation of long (≥10 min) bouts of MVPA was positively associated with upper limbs BMD ($R = 0.220$, $p < 0.05$).

Partial Correlations

In relevant cases above with significant bivariate correlations, known covariates were then factored into the association using partial correlations, resulting into adjusted correlation coefficient ($R_{adj}{}^{n}$).

Men Only Partial Correlations

In men, when adjusting for covariates (i.e., BMI and A:G ratio) the partial associations between upper limbs BMD and short duration SB bouts count, and breaks in SB were nullified ($p > 0.05$) however the correlation against sleep remained ($R_{adj}{}^{n} = -0.321$, $p = 0.012$). When adjusting for covariates (i.e., BMI and total fat), the association between lower limbs BMD against many PB parameter was strengthened including MVPA in % PA bout time ($R_{adj}{}^{n} = 0.436$, $p = 0.001$), LIPA in % PA bout time ($R_{adj}{}^{n} = -0.494$, $p < 0.001$), mean PA bout length ($R_{adj}{}^{n} = -0.476$, $p < 0.001$), PA bouts length in min ($R_{adj}{}^{n} = -0.354$, $p = 0.006$), long SB bouts count ($R_{adj}{}^{n} = 0.321$,

TABLE 4 | Bone health and general physical behavior.

	Sleeping (hours/24 h)	SB (hours/24 h)	Standing (hours/24 h)	LIPA (hours/24 h)	MVPA (hours/24 h)	SB (% waking hours)	Standing (% waking hours)	LIPA (% waking hours)	MVPA (% waking hours)
(A) Bivariate correlation analysis of BMD and General Physical Behaviour in men									
Ribs	−0.203	0.097	−0.181	0.085	−0.068	0.035	−0.208	−0.006	−0.045
Spine	**−0.238***	0.133	−0.127	0.027	0.001	0.060	−0.149	0.013	−0.032
Pelvis	**−0.307*** **(R_{adj}^{n} = −0.361**)**	0.104	0.101	−0.031	0.136	0.008	0.093	−0.052	0.052
Upper_limb	**−0.374**** **(R_{adj}^{n} = −0.321*)**	0.077	−0.127	0.119	0.169	−0.035	−0.162	0.036	0.115
Lower_limb	−0.202	−0.034	−0.079	0.123	0.177	−0.102	−0.092	0.106	0.132
Total	−0.171	−0.030	−0.017	0.125	0.210	−0.088	−0.033	0.119	0.153
Z_score	**−0.261***	0.040	−0.054	0.146	0.137	−0.024	−0.071	0.112	0.068
(B) Bivariate correlation analysis of BMD and General Physical Behaviour in women.									
Ribs	−0.042	0.185	−0.090	−0.156	−0.147	0.190	−0.102	−0.180	−0.162
Spine	0.090	**0.233*** (R_{adj}^{n} is *n.s.*)	−0.130	**−0.225*** (R_{adj}^{n} is *n.s.*)	**−0.256*** (R_{adj}^{n} is *n.s.*)	**0.263*** (R_{adj}^{n} is *n.s.*)	−0.128	**−0.225***	**−0.248***
Pelvis	0.075	0.172	−0.004	**−0.242*** (R_{adj}^{n} is *n.s.*)	−0.091	0.145	−0.012	**−0.242*** (R_{adj}^{n} is *n.s.*)	−0.111
Upper_limb	−0.067	0.131	−0.143	**−0.230*** (R_{adj}^{n} is *n.s.*)	0.074	0.093	−0.158	**−0.241*** (R_{adj}^{n} is *n.s.*)	0.037
Lower_limb	0.054	**0.272*** **(R_{adj}^{n} = 0.260***	−0.140	**−0.320**** **(R_{adj}^{n} = −0.245*)**	−0.207	**0.250*** **(R_{adj}^{n} = 0.277*)**	−0.148	**−0.321****	−0.215
Total	0.154	**0.317**** (R_{adj}^{n} is *n.s.*)	−0.150	**−0.337**** (R_{adj}^{n} is *n.s.*)	**−0.350**** (R_{adj}^{n} is *n.s.*)	**0.359**** **(R_{adj}^{n} = 0.222*)**	−0.136	**−0.330****	**−0.342****
Z_score	**0.225*** (R_{adj}^{n} is *n.s.*)	0.185	−0.090	**−0.258*** (R_{adj}^{n} is *n.s.*)	**−0.224*** (R_{adj}^{n} is *n.s.*)	0.208	−0.085	**−0.236*** (R_{adj}^{n} is *n.s.*)	−0.207

*Analysis was performed between each variable and specific bone BMD site by sex *P < 0.05; **P < 0.01 (one-tailed); correlation coefficients are presented. Plain for Pearson and in shaded gray boxes for Spearman' Rho. R_{adj}^{n} are the partial correlation results. n.s. is non-significant.* ***(A)*** *Bivariate correlation analysis of BMD and general physical behavior in men.* ***(B)*** *Bivariate correlation analysis of BMD and general physical behavior in women.*

TABLE 5 | Bone health and sedentary behavior.

	Breaks in SB (*n*)	<5 min SB bout (*n*)	≥5 min SB bout (*n*)	Mean SB bout length (min)	Alfa	W50% (min)
Ribs	**0.282***	0.113	**0.349****	−0.136	−0.066	−0.171
Spine	0.156	0.102	0.192	−0.041	−0.157	−0.014
Pelvis	0.205	0.130	0.158	0.003	−0.117	0.032
Upper_limbs	**0.403**** (R_{adj}^n is *n.s.*)	**0.238*** (R_{adj}^n is *n.s.*)	0.088	−0.082	−0.140	−0.078
Lower_limbs	0.222	−0.032	**0.290* (R_{adj}^n = 0.321*)**	0.018	−0.109	−0.011
Total	**0.330****	0.115	**0.373****	−0.082	−0.221	−0.095
Z_score	**0.296***	0.161	**0.283***	−0.042	−0.167	0.020
Ribs	**0.266*** (R_{adj}^n is *n.s.*)	**0.328**** **(R_{adj}^n = 0.251*)**	0.082	−0.181	−0.035	**−0.224*** (R_{adj}^n is *n.s.*)
Spine	0.158	0.217	−0.011	−0.118	−0.019.	−0.141
Pelvis	**0.232*** (R_{adj}^n is *n.s.*)	0.208	0.172	−0.130	0.056	−0.131
Upper_limbs	0.089 *n.s.*	**0.230***	−0.060	−0.123	−0.002	−0.128
Lower_limbs	**0.299*** (R_{adj}^n is *n.s.*)	**0.334**** (R_{adj}^n is *n.s.*)	0.137	−0.155	0.058	−0.157
Total	0.202	0.216	0.100	−0.062	−0.035.	−0.105
Z_score	0.148	0.221	0.003	0.016	−0.091	0.044

*Analysis was performed between each variable and specific bone BMD site by sex *P < 0.05; **P < 0.01 (one-tailed); n.s. is non-significant; correlation coefficients are presented. Plain for Pearson and in shaded gray boxes for Spearman' Rho. R_{adj}^n are the partial correlation results.* ***(A)*** *Bivariate correlation analysis of BMD and sedentary behavior in men.* ***(B)*** *Bivariate correlation analysis of BMD and sedentary behavior in women.*

$p = 0.012$). Together, this suggests that the associations with upper and lower limbs BMD and many PBS parameters were not just a product of anthropometric factors. Next, the partial correlation between pelvis vs. sleep, controlling for BMI still maintained a significant impact of this PB ($R_{adj}^n = -0.361$, $p = 0.005$). Finally, the partial correlation between spine vs. mean PA bout length, controlling for BMI still maintained a significant impact of this PB ($R_{adj}^n = -0.246$, $p = 0.042$).

Women Only Partial Correlations

In women, when adjusting for covariates (age, BMI and total fat), correlations between upper limb BMD and LIPA in % waking hours, number of short SB, and total weekly time in prolonged MVPA were all nullified ($p > 0.05$).

With lower limbs BMD, adjusting for covariates (i.e., BMI, total Fat and A:G ratio), the associations (adjusted correlation coefficient or R_{adj}^n) against SB (in hours per day; $R_{adj}^n = 0.260$, $p = 0.028$, and as a percent of waking hours; $R_{adj}^n = 0.277$, $p = 0.020$) and against LIPA in hours per day ($R_{adj}^n = -0.245$, $p = 0.028$) all remained. However, the associations between lower limb BMD and the previously significantly associated number of breaks in SB, number of short SB, number of PA bouts, all disappeared ($p > 0.05$).

With pelvic BMD adjusting for covariates (i.e., BMI, total fat and A:G ratio) partial correlations against LIPA (in hours per day, and in % waking hours), breaks in SB and number of PA bouts all disappeared. The same applied for the spine when adjusting the observed correlations for covariates (i.e., BMI and total fat). This disappearance of correlations between measures of PB patterns with BMD points to the importance of anthropometry and/or body composition in women for the BMD in these sites.

For the ribs the association between BMD with number of short bouts of SB ($R_{adj}^n = 0.251$, $p = 0.031$) and total weekly duration in PA ($R_{adj}^n = 0.327$, $p = 0.007$) remained after adjusting for covariates (i.e., BMI and total fat). However, the partial correlations between rib BMD and number of breaks in SB, W50% and number of PA bouts had disappeared.

For total BMD the partial correlations against SB in % waking hours ($R_{adj}^n = 0.222$, $p = 0.050$) remained after adjusting for covariates (i.e., BMI and total fat), but the associations against SB in hours per day, LIPA in hours per day, and MVPA in hours per day all disappeared.

Finally, the partial correlations of total BMD Z-score against sleep, LIPA in hours per day, LIPA in % waking hours, MVPA in hours per day and mean PA bout length became all statistically non-significant after adjusting for covariates (i.e., age, BMI and total fat).

Z_{PB} Score Graphic Synthesis of Physical Behavior Patterns by Bone Health Clinical Sub-Groups

None of the parameters of PB consistently correlated with BMD. To evaluate whether PB parameters differed dependent on bone health we classified people as having healthy, osteopenic, and osteoporotic bones, by the T-scores (see methods). We expressed all 26 PB parameters as dimensionless Z_{PB}-scores and drew radar graphs to determine whether any patterns in PA were associated with bone health status. This analysis was carried out on lower limbs BMD and upper limbs BMD, separately for men and women.

In the men, the T-score data results revealed none as osteoporotic, 4 men as osteopenic and 47 men as having a healthy skeleton. In the upper limbs this translated to BMDs of 0.77 ± 0.05 g/cm^2 and 0.90 ± 0.07 g/cm^2 in the osteopenic and healthy group ($p < 0.001$), respectively, and in the lower limbs BMDs of 1.09 ± 0.07 g/cm^2 and 1.32 ± 0.16 g/cm^2 ($p = 0.001$).

The PB of each of the men's clinical groups are illustrated in **Figure 1A** for T-scores, **Figure 1B** for upper limbs BMD and **Figure 1C** for lower limbs BMD. Each figure provides a

TABLE 6 | Bone health and physical activity.

	PA bouts (*n*)	PA bouts (total min)	Mean PA bout length (min)	SB (%PA bout time)	Standing (%PA bout time)	LIPA (%PA bout time)	MVPA (%PA bout time)	≥10 min MVPA bouts (total min)	≥10 min MVPA (*n*)	Sporadic MVPA (min)	Total week ≥ 10 min MVPA (min)
(A) Bivariate correlation analysis of BMD and Physical Activity in men											
Ribs	**0.282***	−0.048	−0.216	−0.067	0.118	−0.204	0.049	−0.106	−0.096	0.063.	−0.106
Spine	−0.156	−0.178	**−0.248*** (R_{adj}^{n} = **−0.246***)	−0.010	−0.063.	−0.139	0.122	−0.155	−0.095	−0.029	−0.153
Pelvis	0.205	−0.083	−0.181	−0.212	−0.075	−0.115	0.080	−0.052	−0.070	0.095	−0.062
Upper_limbs	**0.238***	−0.050	−0.192	0.028	0.021	**−0.289***	0.169	−0.025	−0.029	0.081	−0.028
Lower_limbs	0.222	**−0.254*** (R_{adj}^{n} = **−0.354****)	**−0.396**** (R_{adj}^{n} = **−0.476*****)	0.044	−0.111	**−0.414**** (R_{adj}^{n} = **−0.494*****)	**0.285*** (R_{adj}^{n} = **0.436****)	0.050	0.035	−0.020	0.049
Total	**0.330****	−0.231	**−0.445****	0.049	−0.064	**−0.338****	0.201	−0.057	−0.058	0.055	−0.060
Z_score	**0.296***	−0.176	**−0.362****	−0.020	−0.024	−0.195	0.071	−0.085	−0.112	0.042	−0.088
Ribs	**0.266*** (R_{adj}^{n} is *n.s.*)	**0.265*** (R_{adj}^{n} **0.327****)	0.059	−0.085	0.085	0.028	−0.058	0.153	0.140	−0.083	0.157
Spine	0.158	0.184	0.087	−0.093	0.019	0.059	−0.037	0.013	−0.005	−0.062	0.021
Pelvis	**0.232*** (R_{adj}^{n} is *n.s.*)	0.076	−0.090	−0.022	−0.032	−0.020.	0.030	0.052	−0.003	0.027	0.068
Upper_limbs	0.089	0.200	0.162	−0.054	0.153	−0.016	−0.082	0.209	0.170	0.070	**0.220*** (R_{adj}^{n} is *n.s.*)
Lower_limbs	**0.299*** (R_{adj}^{n} is *n.s.*)	0.194	−0.036	−0.009	0.095	0.135	−0.167	0.131	0.115	0.016	0.146
Total	0.202	0.069	−0.082	−0.079	0.075	0.152	−0.139	0.079	0.049	−0.081	0.085
Z_score	0.148	−0.139	**−0.235*** (R_{adj}^{n} is *n.s.*)	−0.050	0.103	0.110	−0.098	−0.005	−0.058	−0.192	−0.006

*Analysis was performed between each variable and specific bone BMD site by sex *P < 0.05; **P < 0.01; ***P < 0.001 (one-tailed); n.s. is non-significant; correlation coefficients are presented. Plain for Pearson and in shaded gray boxes for Spearman' Rho. R_{adj}^{n} are the partial correlation results.* ***(A)*** *Bivariate correlation analysis of BMD and physical activity in men.* ***(B)*** *Bivariate correlation analysis of BMD and physical activity in women.*

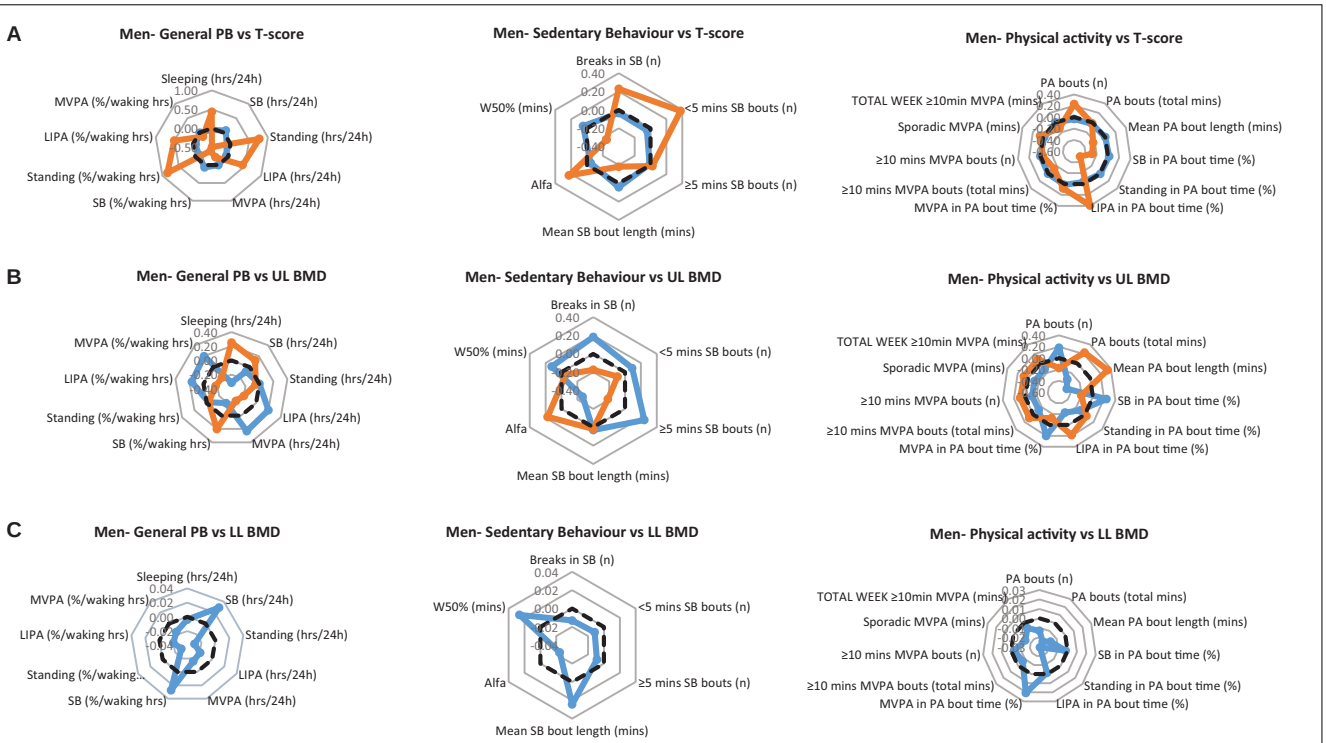

FIGURE 1 | Physical behaviors (PB) in healthy (blue) and osteopenic (orange) men **(A)** as defined by the whole body T-score, **(B)** upper limbs BMD T-score, and **(C)** lower limbs BMD T-score. The radar graphs depict in separate panels (from left to right), general physical behaviors, sedentarism-specific behaviors, and physical activity-specific behaviors. Sample mean is shown in a black dashed line. PB data shown are computed Z-scores.

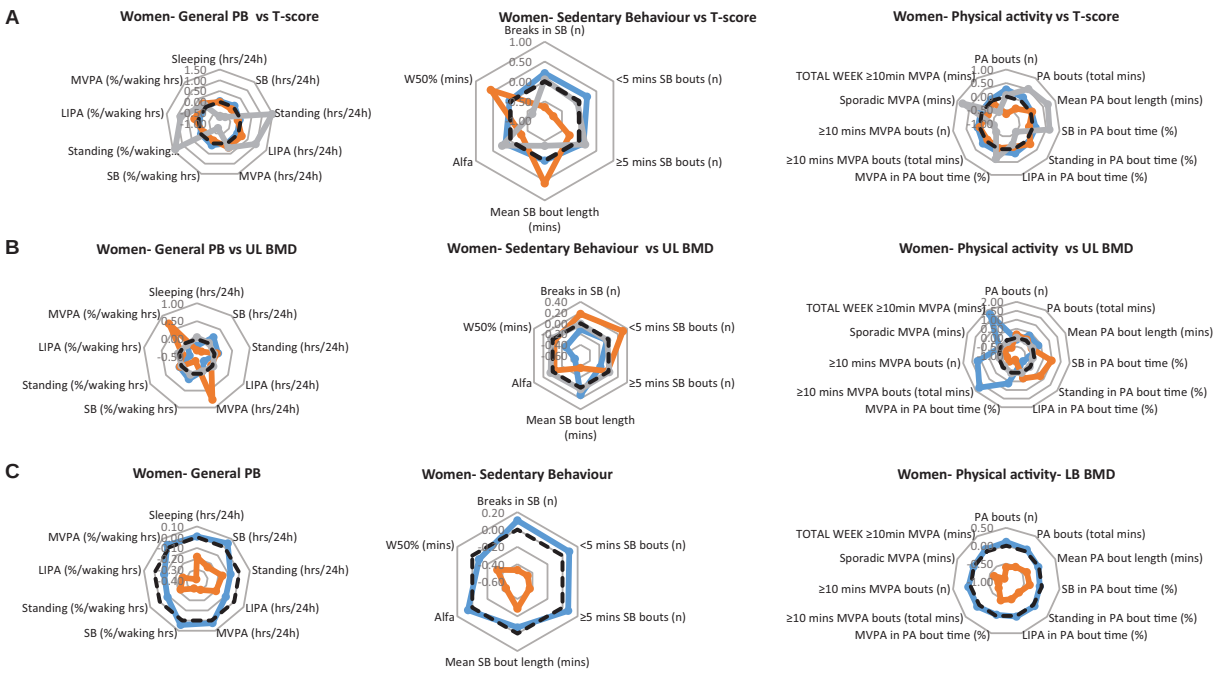

FIGURE 2 | Physical behaviors (PB) in healthy (blue), osteopenic (orange), and osteoporotic (gray) women **(A)** as defined by the whole body T-score, **(B)** upper limbs BMD T-score, and **(C)** lower limbs BMD T-score. The radar graphs depict in separate panels (from left to right), general physical behaviors, sedentarism-specific behaviors, and physical activity-specific behaviors. Sample mean is shown in a black dashed line. PB data shown are computed Z-scores.

profile for general PB, SB specific PB and PA specific PB. We especially take note of the lower limbs, since these arguably are the bone sites most likely to be impacted upon by the tracked PB. Here, the men with healthy bones tended to show higher values for SB (hours/day and as % of waking hours) compared to the osteopenic group. However, the healthy lower limb bones on men were associated with significantly lower Standing (hours/day and as % waking hours), significantly lower LIPA (hours/day and as % waking hours) and significantly lower MVPA (hours/day and as % waking hours). Healthy lower limbs BMD were also associated with greater W50% and mean SB bout length (min). More in line with our expectations, however, healthy lower limbs BMD was associated with greater MVPA amount (as % PA bout time).

In the women, the T-score data results revealed 4 women as osteoporotic, 16 women as osteopenic and 40 women as having a healthy skeleton. In their upper limbs this translated to BMDs: osteoporotic: 0.61 ± 0.03 g/cm^2; osteopenic: 0.66 ± 0.04 g/cm^2; healthy: 0.74 ± 0.10 g/cm^2 (all comparisons $p < 0.001$). In their lower limbs this translated to BMDs: osteoporotic: 0.86 ± 0.04 g/cm^2; osteopenic: 0.93 ± 0.08 g/cm^2; healthy 1.13 ± 0.17 g/cm^2 (all comparisons $p = 0.001$).

The PB of each of the women's clinical groups, are illustrated in **Figure 2A** for T-scores, **Figure 2B** for upper limbs BMD and **Figure 2C** for lower limbs BMD. Each figure provides a profile for general PB, SB specific PB and PA specific PB. As with the men's data, we especially take note of the lower limbs. Here, the women with healthy bones tended to show higher values for all general PBs compared to the osteopenic group (there was no osteoporotic lower limbs group in the women sample). Interestingly, the same was also true for all SBs as well as PA behaviors.

In addition, it should be noted that in the women as in the men, the differences in PB between groups tended to be within 1 standard deviation, demonstrating that in absolute terms, the groups had very similar PBs in many cases.

In men, there were no statistical significant differences in general PBs ($P > 0.05$), nor in SB specific parameters ($P > 0.05$) between those with healthy and those with osteopenic bones. However, SB in % PA bout time was less ($p = 0.037$), MVPA in % PA bout time was more ($p = 0.031$) in osteopenic than in normal bone men (**Figure 1A**, right panel).

In women, there was a main effect of group for standing [in hours per day ($p = 0.016$), and in % waking hours ($p = 0.022$)], LIPA (in hours per day; $p = 0.037$), number of breaks in SB ($p = 0.010$), number of short SB bouts ($p = 0.002$), W50% ($p = 0.026$) and number of PA bouts ($p = 0.010$). This was reflected by longer standing, LIPA (**Figure 2A**, left panel), and larger number of PA bouts (**Figure 2A**, right panel) in the osteoporotic than osteopenic and healthy women. The number of breaks of SB was larger and the number of SB bouts smaller in osteoporotic and healthy than osteopenic women, while the W50% was largest in the osteopenic and smallest in the osteoporotic women (**Figure 2A**, middle panel).

DISCUSSION

The current study quantified habitual PB (i.e., total SB and PA, as well as patterns) to establish any association with bone health. It was hypothesized that (1) individuals engaging more frequently in light-intensity and moderate-vigorous exercise demonstrate better bone health than their less active counterparts; (2) greater SB time is associated with poorer bone health; (3) individuals breaking prolonged bouts of SB more frequently display better bone health.

The main observation of the present study is that in men out of the possible 182 correlations, 12 supported our hypotheses, 12 went against expectations and the rest (i.e., 158) showed no association. In women, one correlation appeared to support our hypotheses, 12 went against face value expectations and the rest (i.e., 169) showed no association. There were also no expected differences in PB between people with osteoporotic, osteopenic or healthy bones. These observations thus suggest that in contrast to observations of bone loss during bed rest, even SB in older people does not aggravate the aging-related bone loss.

Comparing between T-score categories in men, it transpired that only SB in % PA bout time, and MVPA in % PA bout time differed between healthy vs. osteopenic men. Comparing the PBs of osteoporotic vs. osteopenic vs. healthy T-scores women, revealed group differences in standing (in hours per day, and in % waking hours), in LIPA (in hours per day), in the number of breaks in SB, in the number of short SB bouts, in W50% and in the number of PA bouts.

Bone homeostasis has been demonstrated to become compromised due to a significant age-decline in Vitamin D (>60 years of age), through reduced dietary intake, and decreased exposure to sunlight attributed to mentioned increases in SB (Riggs et al., 1982; Lips, 2001) and indoor activities. Subsequent effects include an over secretion of the parathyroid hormone (hyperparathyroidism), a proven contributor to the loss of cortical bone, causing calcium to be released from a number of reservoirs within both bone and kidney and further depleted and excreted after renal filtration (Riggs et al., 1982; Lips, 2001). In terms of lifestyle, previous research suggests that prolonged bed rest leads to decreased BMD, alongside a prevalence of biochemical markers of bone resorption (NTx), and increased urinary calcium, all factors linked to a causal relationship between PB and bone characteristics.

Physical Activity and Bone Health

As described above, in contrast to our expectation the level of PA had a negligible effect on BMD. If anything there was a sex bias whereby men tended to show positive links with PA whereas surprisingly, there tended to be a negative association between several BMD data and PA in women.

Total PA for both sexes, and especially MVPA in men and to a lesser extent in women, were in fact significant predictors of bone health. Our findings, even in this group that seldom engaged in MVPA (19.49 ± 5.38% vs. 18.68 ± 5.50% of waking hours, respectively, in men and in women) are in agreement with previous studies reporting greater femoral BMD following engagement in habitual daily physical activities such as walking

and stair climbing (Cummings et al., 1995; Kohrt et al., 1997). Overall, it would seem that any impact of PA may be more likely to be targeted to bone sites close to joints responsible for postural balance and ambulation as these experience regular absorption of GRFs to induce structural increases (Cummings et al., 1995; Kohrt et al., 1997). It is also interesting to note that it is possible that the significant negative associations between LIPA (∼12% of waking hours) and bone health may be a reflection of the overall low engagement in PA for this age group, hence any other lifestyle factors would have been likely to override its effects.

Our findings are also consistent with a report by others (Humphries et al., 1999), who concluded that the loss of bone may be of greater magnitude when compared to its formation during older age, more so in the women. Thus engagement in MVPA and LIPA may not be enough to prevent changes in bone metabolism solely; less time spent being sedentary may also be needed. Alternatively, findings may be attributed to the possibility that these older participants exhibited a number of other factors deleterious to bone formation and or maintenance. These could range, as often is the case with normal aging, from poor calcium retention, Vitamin D deficiency, or impaired parathyroid hormone secretion (Riggs et al., 1982; Cavanaugh and Cann, 1988; Humphries et al., 1999; Manolagas, 2000; Min et al., 2000; Chastin et al., 2014), and/or body composition/sub-optimal food intake (Tomlinson et al., 2019). Thus, any MVPA and LIPA in the habitual lifestyle of our female cohort in particular, was simply not sufficient to overcome these other factors. The fact that MVPA had a negative association with bone health in two cases in women (ribs BMD and total BMD), is also thought provoking. It could be that the MVPA-induced micro-damage may be slightly larger than the regeneration resulting in overall bone loss. Similarly, it is also highly likely that the activities of our participants did not reach the impact (acceleration $>$ 4.2 g) or speed (10 km/h) purported as threshold needed to achieve sufficient stimulus for bone formation (Deere et al., 2012). Indeed, it has been shown that while master sprinters had a larger BMD than age-matched non-athletes, no such benefit was seen in even endurance master athletes (Piasecki et al., 2018). Also in line with the idea that a threshold of acceleration is required, accelerometer data in a sample of master athletes (Deere et al., 2016) and highly active postmenopausal women (Hannam et al., 2017) is shown to exhibit higher *Y*-axis peak accelerations in those compared with generally sedentary sex- and age-matched controls.

Sedentary Behavior and Bone Health

In some cases, bone health was positively associated with the number of breaks in SB, but only within the cohort of men. Findings concur with previous studies whereby immobilization of the ambulatory limbs induced hormonal responses responsible for disruption of calcium metabolism necessary for bone formation (Kim et al., 2003, 2010; Smith et al., 2003; Zwart et al., 2009). Additionally, as biochemical markers of resorption (NTx, urinary calcium) are reported to elevate significantly after as few as 6 days of bed rest in a young healthy cohort; the assumption could be made that amongst a consistently sedentary, older cohort, these endocrine markers could also exist and be emphasized. Future studies should aim to collect serum and/or urine sample to describe any link between endocrine bone factors and SB. Indeed the hypothesis would be that where habitual loading is low, and hence the skeletal system is exposed to sub-optimal stress and strain, this would lead to less stimulation of bone formation and hence, in a shift from formation to resorption.

In contrast, high sleep time was seen to be detrimental toward BMD, with a negative correlation being established with several BMD sites, in the men but not in women. At the morphologic level, previous bed rest studies suggested that the hypoactivity-induced decreased BMD in men is accompanied by reductions in cortical area and cortical thickness, but increases in periosteal perimeter and trabecular area (Belavy et al., 2011).

It was surprising that high numbers of prolonged SB bouts were associated with better bone health. This may be partially explained by the BMI of these older participants being predominantly in the 'overweight category.' This has previously been reported to contribute to a higher BMD (Nordman et al., 2018). Increased loading, associated with the higher BMI, onto the skeleton is not necessarily the only route for this effect (Andersen et al., 2014).

In women, a higher number of short SB bouts and elevated total sedentary time ($>$60% of waking hours) were associated with a larger BMD. It remains unclear why the ribs region are particularly sensitive to disuse (Zerwekh et al., 1998). Indeed our conjecture is in line with the variation in the single Mechanostats setting, which favors the existence of different bone loading thresholds for different populations and bone sites (Skerry, 2006).

While at first glance these data may seem at odds with the benefits of loading for BMD (Frost, 1987a,b) it should be noted that the rib BMD in women was also positively related to the total weekly duration in PA. These apparently conflicting associations can be reconciled when one considers that a larger number of short SB bouts must imply a more frequent interruption of SB and hence a higher total PA. This and the fact that PA requires enhanced ventilation then results in enhanced loading of the ribs by the respiratory muscles and hence explain the positive relationship of rib BMD with both weekly PA duration and the number of short SB bouts. This then suggests that, at least in women, the rib BMD is positively influenced by PA. Alternatively, we would propose that a forward stooped posture commonly adopted by older persons, whilst walking and/or sitting, may be the cause for this regional effect. Indeed biomechanically speaking, the trunk region is kyphotic, and forward stooping would accentuate this curvature. A forward stoop would increase (forward) shear forces between thoracic vertebrae, and thus place additional stress/strain on the bone structures including the ribs, in this hyper-kyphotic position including para-spinal muscles and ligaments, thereby increasing the forces acting on the vertebrae (i.e., at their attachments).

Study Limitations

A limitation for the present study was the lack of inclusion of detailed dietary parameters. Indeed while the DEXA scanning procedure includes a questionnaire on habitual dairy products intake, smoking habits and alcohol consumption, the details

are not sufficiently refined (given these are self-reported data) to reliably include in the regressions. Precise data on a number of other factors that influence bone turnover would be ideal, including vitamin C and vitamin D intake, years post-menopause, family history (Riggs et al., 1982; Cummings et al., 1995; Chastin et al., 2014), and macronutrients diet composition and caloric intake (Tomlinson et al., 2019). Thus, future studies should take these into account in order to increase the granularity of our understanding of the unique impact of PB on bone health.

The paucity of positive associations between PA and bone site BMD, may be linked to a threshold of PA to affect bone. As we have discussed in the text above, it is possible that in their daily activities, this older age cohort (in carrying out PA at self-selected PA intensities and frequencies) may have self-selected activities inadequate to reach a key physiological threshold required to promote bone formation (Lanyon, 1992; Kohrt et al., 1997; Humphries et al., 1999). In addition, we note that running such a large number of correlations potentially increased type II errors, which was somewhat mitigated by looking at each sex separately and not overlapping our hypotheses. To build on our present work, we recommend that a future study with a larger sample size (including 15 participants for each PB outcome), utilizes a multiple linear/temporal substitution regression approach and estimates the power of such regressions based on the attained explained variance.

Last, we utilized one current week of PB and inferred this was a reflection of the long-medium term pattern, and this may not necessarily be true. However, to make the PB data as much as possible representative for the usual PB (1) we asked the participants to continue their daily life as usual and (2) included both weekdays and weekends that typically differ in PB even in retirees (McCormack et al., 2010). In future studies, PB may be monitored at two time points, separated by at least 6 months, to also adjust for potential seasonal variations in PB.

CONCLUSION

In this sample of community-dwelling elders, PB is clearly able to distinguish one clinical sub-group from another. This is evidenced through bivariate correlations as well as group comparisons of overall PB (Z_{PB}-scores). Indeed the latter is an approach which is part of the strength of the current study, providing as it does, both a visual and a quantitative representation of the overall PB pattern differences between samples (in our case bone health groups). What is also clear, is the sex specificity of these modulations. In fact, the Mechanostat theory (Frost, 1987a,b) does not apply indiscriminately: it is not necessarily where we see more loading that we may infer a healthier bone profile.

ETHICS STATEMENT

Participants were recruited by word-of-mouth from a number of national organizations and local clubs (including the University of the Third Age (U3A), Rotary, Age United Kingdom, local golf clubs). Hundred and twelve adults (men, N = 51/women, N = 61) volunteered to participate in this study. All were aged between 57 and 89 years (average ± SD = males 73.6 ± 6.2 years, females 71.6 ± 6.4 years) and were of differing self-reported PA status. Full ethical approval was received through the Manchester Metropolitan University Ethics Committee prior to experimentation, and informed written consent was obtained from each participant.

AUTHOR CONTRIBUTIONS

GO-P, CM, and HD designed the research. JW and DR conducted the research. JW, DR, CD, and GO-P analyzed the data. CD, GO-P, and HD wrote the manuscript and this was reviewed by all co-authors. GO-P has primary responsibility for final content. All authors read and approved the final manuscript.

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Association of Physical Activity With Telomere Length Among Elderly Adults - The Oulu Cohort 1945

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Introduction: Physical activity (PA) has been associated with telomere shortening. The association of PA intensity or volume with telomere length (TL) is nonetheless unclear. The aim of our study was to investigate the associations of exercise intensity and volume with TL in elderly adults from Northern Finland (65° latitude North).

Methods: Seven hundred elderly subjects born in 1945 in the Oulu region were investigated. PA was measured during a 2-week period with a wrist-worn accelerometer. In addition, a questionnaire was used to assess sedentary time and to achieve a longitudinal PA history and intensity. Relative telomere lengths (RTL) were determined from frozen whole blood samples using a qPCR-based method.

Results: Relative telomere lengths were significantly longer in women than men and negatively correlated with age in both genders (men $r = -0.210$, $p = 0.000$, women $r = -0.174$, and $p = 0.000$). During the 2-week study period, women took more steps than men ($p = 0.001$), but the association between steps and RTL was only seen in men ($p = 0.05$). Total steps taken ($r = 0.202$ and $p = 0.04$) and sedentary time ($r = -0.247$ and $p = 0.007$) significantly correlated with RTLs in 70-year old subjects. Moderate PA was associated with RTL in subjects with the highest quartile of moderate PA compared to the three lower quartiles (p-values: 0.023 between 4th and 1st, 0.04 between 4th and 2nd, and 0.027 between 4th and 3rd) in the 70-year old subjects.

Conclusion: Women had longer RTL and a higher step count compared to men. However, exercise volume and RTL correlated positively only in men. Surprisingly, age correlated negatively with RTL already within an age difference of 2 years. This suggests that telomere attrition rate may accelerate in older age. Moderate physical activity at the time of study was associated with RTL.

Keywords: physical activity, elderly, telomeres, objective measurements, step counts, questionnaires

INTRODUCTION

Telomeres are looped structures located at the end of chromosomes, protecting our genomic DNA from degradation. Telomeric DNA consists of repetitive sequences of TTAGGG, common to all mammals (Aubert and Lansdorp, 2008). Double-stranded structure changes into single-stranded structure, creating a 3′ overhang in the G-rich strand, which is a principal feature in loop formation, hiding the chromosomal ends from the DNA damage repair machinery. Due to the properties of DNA replication, DNA synthesis cannot proceed to the end of the chain (end-replication problem), thus shortening of the telomere with each cell division by approximately 50–100 bp occurs (Sanders and Newman, 2013). In addition to telomeric DNA, the six subunit protein complex shelterin is needed for telomere structure and function (Podlevsky and Chen, 2012). Different subunits interact with DNA and telomerase holoenzyme. Telomerase adds TTAGGG repeats onto the chromosomal ends and thus is responsible for telomere length (TL). Telomerase is active in germ and stem cells, while its activity diminishes in somatic tissues, leading to telomere shortening with each cell division (Hayflick limit). At least a 400 bp of the telomeric repeat sequence is needed for maintaining a functional telomere, but experiments with cancer cell lines have demonstrated that TL less than 1 kb is sufficient to induce senescence (de Lange, 2009). Human TLs are between 10 and 15 kb at birth, and then gradually decline.

Telomere length at birth is similar in both genders, but women have longer telomeres later in life (Seifarth et al., 2012). Estrogen and higher compatibility between mitochondrial and genomic DNA have been associated with higher TL in women. TL has been shown to be hereditary from the paternal side (Nordfjäll et al., 2005). Furthermore, previous work has shown that high stress levels (both psychological and oxidative, determined via the 10-item Perceived Stress Scale and isoprostanes per milligram of creatinine/vitamin E) are associated with shortened TL (Epel et al., 2004). Seventy-five minutes of vigorous exercise weekly was found to be associated with longer telomeres when experiencing psychological stress (The 10-item Perceived Stress Scale) (Puterman et al., 2010). These findings suggest a complex network influencing the maintenance and integrity of telomeres, which includes genetic, lifestyle, psychological and physiological factors.

Several studies have shown that low PA is associated with telomere shortening (Ludlow et al., 2008; Puterman et al., 2010; Ludlow and Roth, 2011; Bojesen, 2013; Weischer et al., 2014; Shadyab et al., 2017a,b; Williams et al., 2017). Tucker (2017) demonstrated that sedentary people were 9 years pre-aged on the cellular levels (based on shorter TL) compared to people in the high PA activity group. Conversely, Savela et al. (2013) reported that subjects exercising with moderate intensity (MPA) had the longest telomeres. At the cellular level an age difference of 4 to 6 years was observed between those of moderate compared to those of low intensity PA. Maximal oxygen uptake ($\dot{V}O_2$ max) has been shown to positively correlate with TL (LaRocca et al., 2010; Østhus et al., 2012). Interestingly, however, in extreme endurance athletes (e.g., marathon runners), TL is similar to sedentary subjects, suggesting that excessive training might be harmful (Mathur et al., 2013). Training hours and years of practice at a professional level correlated negatively with TL in professional endurance runners (Rae et al., 2010). The same association was observed in competitive powerlifters; the TL in their *vastus lateralis* correlated inversely with the subject's record in squat and deadlift (Kadi et al., 2008). These findings suggest an inverted U-shaped relationship between PA intensity and TL, with both high and low PA levels associated with shortened TL. Shadyab et al. (2017a) showed that greater amounts of moderate-to-vigorous PA were associated with longer telomeres in elderly women. Among less physically active older women, sedentary time was associated with shorter TL (Shadyab et al., 2017b). Shorter telomeres are associated with limitations in physical functioning compared to subjects with long telomeres in elderly European populations (Rojas et al., 2018).

In addition, production of reactive oxygen species (ROS) contributes to increased DNA damage, apoptosis and senescence (Kawanishi and Oikawa, 2004). Especially, ROS have been shown to influence the central 5′-GGG-3′ guanine segment, abundant in telomeric DNA (Bojesen, 2013; Arsenis et al., 2017). Importantly, regular PA has been showed to reduce ROS levels (He et al., 2016). Based on the studies mentioned above, the relationship between PA amounts and intensities and TL is still unclear, especially in the older age groups. The aim of our study was to assess the associations of volume and intensity of PA with TL among older adults in a cross-sectional study in the Oulu cohort 1945 from Northern Finland. We hypothesized that higher amounts of PA would be associated with longer TL.

MATERIALS AND METHODS

Study Population

The study population was based on a health survey conducted in 2002 among all persons born in 1945 and living in the City of Oulu, Finland (120 000 inhabitants, 65°01′ N, 25°28′ E) (Juuti et al., 2008). 904 of those were invited for a follow up study during the years 2013–2015 (**Figure 1**). 204 declined to participate. The data collection took place over 2 years, resulting in a maximum age difference of 2 years within the study population. The study was approved by the ethical committee of the Northern Ostrobothnia Hospital District and has been carried out according to the National legislation and guidelines and the declaration of Helsinki. All subjects gave written informed consent in accordance with the Declaration of Helsinki. The data collection has been previously described (Metsämarttila et al., 2018). Covariates presented in **Table 1** were collected during visits in 2013–2015. Age, gender, education, smoking and hypertension medication usage were assessed by a questionnaire. Weight, height, waist circumference and blood pressure were measured and blood samples drawn by a licensed nurse. Body mass index (BMI) was calculated as weight in kilograms divided by height meters squared. Cholesterol, triglycerides, C-reactive protein (CRP), glycated hemoglobin A1c (HbA1c), fasting glucose, fasting insulin, and relative telomere length (RTL) were analyzed from the blood samples. Homeostatic model

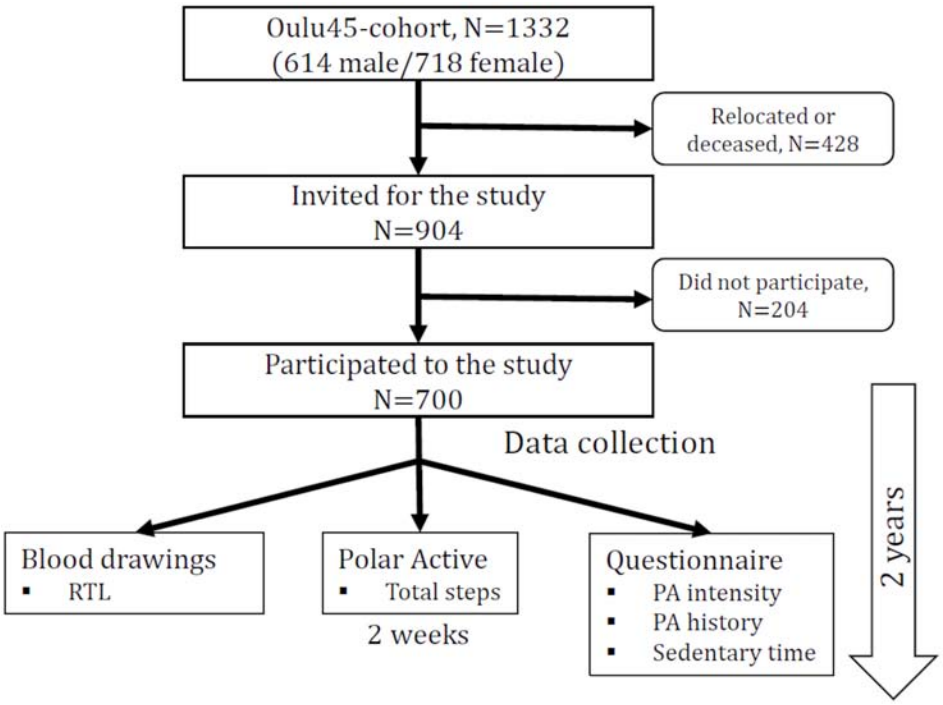

FIGURE 1 | Flow chart of the study.

TABLE 1 | Anthropological and biochemical characteristics of the study population.

		Total	Men	Women	*p*-value[a]	*p*-value[b]
Number of subjects		700	296 (42.3%)	404 (57.7%)	–	0.0975
Age (years)		68.9 ± 0.6	68.9 ± 0.5	68.9 ± 0.6	0.595	< 0.0001*
Education	Level	–	–	–	–	0.468
	1	94 (13.4%)	40 (13.6%)	54 (13.2%)	–	–
	2	167 (23.9%)	76 (25.7%)	91 (22.7%)	–	–
	3	155 (22.1%)	59 (19.9%)	96 (23.7%)	–	–
	4	159 (22.7%)	54 (18.0%)	105 (26.0%)	–	–
	5	125 (17.9%)	67 (22.8%)	58 (14.5%)	–	–
Smoker		90 (12.8%)	41 (15.3%)	44 (11.0%)	0.105	0.3294
Alcohol consumption (g/day)		1.9 ± 4.6	2.9 ± 4.7	1.2 ± 4.5	< 0.0001*	–
BMI (kg/m2)		27.68 ± 4.7	28.0 ± 4.3	27.5 ± 5.1	0.164	0.417
Waist (cm)		94.2 ± 13.7	100.0 ± 12.1	90.0 ± 13.3	< 0.0001*	0.458
SP (mmHg)		143.9 ± 17.5	145.7 ± 16.31	142.6 ± 18.2	0.018*	0.681
DP (mmHg)		85.5 ± 9.6	86.1 ± 9.3	85.1 ± 9.8	0.178	0.085
T/S Ratio		0.8 ± 0.3	0.8 ± 0.2	0.8 ± 0.3	0.1056	–
Total cholesterol (mmol/l)		5.3 ± 1.2	4.9 ± 1.1	5.6 ± 1.3	< 0.0001*	0.776
HDL cholesterol (mmol/l)		1.7 ± 0.5	1.5 ± 0.4	1.8 ± 0.4	< 0.0001*	0.626
Triglycerides (mmol/l)		1.3 ± 0.8	1.3 ± 1.0	1.2 ± 0.6	0.123	0.237
CRP (mg/l)		3.5 ± 9.3	3.2 ± 7.0	3.2 ± 10.9	0.453	0.728
HbA1c (%)		5.9 ± 0.5	5.9 ± 0.6	5.9 ± 0.6	0.9897	0.008*
HOMA-IR		1.9 ± 1.4	2.0 ± 1.5	1.8 ± 1.2	0.0982	0.328
MetSyn		362 (51.7%)	131 (44.1%)	226 (55.9%)	0.2486	–
Hypertension medication		366 (52.3%)	128 (43.3%)	229 (56.7%)	0.046*	0.198

*Means ± standard deviations, numbers of subjects and percent of subjects n(%). a = Significance of the difference between the genders. b = significance of the Pearson correlation between each variable and RTL (T/S Ratio). SP: systolic pressure, DP: diastolic pressure. * p-value < 0.05.*

assessment of insulin resistance (HOMA-IR) was calculated according to the formula: fasting insulin (μl/L) x fasting glucose (nmol/L) / 22.5. The metabolic syndrome (MetSyn) was defined according the new International Diabetes Foundation definition (Alberti et al., 2005).

Activity Measurements

We have used two different approaches to determine the level of physical activity. The detailed description and validation of both objective and subjective physical activity measurements was recently reported (Niemelä et al., 2019). The subjects wore the same wrist-worn accelerometer (Polar Active, Polar Electro, Finland) for 2 weeks to record their habitual physical activity. The device recorded the total amount of steps. In addition, subjects filled out a questionnaire which included questions of their current and past PA frequency, intensity and sedentary time. The intensity and frequency were assessed at four time-points (ages of 15, 30, 50, and current age). At these points light intensity (LPA), MPA and vigorous intensity PA (VPA) was determined. LPA consisted of light cycling, walking, gardening, indoors chores and motorbiking. Brisk walking, calm swimming, ice skating, wood or water carrying, brisk cycling, gymnastics at home, and horseback riding was characterized as MPA. VPA included climbing stairs, rowing/cycling/swimming fast, skiing, shoveling/shoveling snow, and brisk walking in the swamp. Subjects were asked to fill in separately, how many times they did these three types of exercise in a week at each point of their lives which were then divided into quartiles for analysis. The quartiles were defined according to how many times a week the subjects engaged in different activities (LPA, MPA, and VPA separately). Time spent sitting in different situations (e.g., at work, commuting, and watching TV) were used to compose the total daily sedentary time during a normal weekday (**Supplementary Table S1**).

Relative Telomere Length Determination

DNA was isolated from frozen whole blood samples using the Nucleospin DNA blood kits (MACHEREY-NAGEL GmbH & Co., KG, Germany) according to manufacturer's instructions with minor modifications in protocol such as increasing lysis incubation time from 10 to 30 min. RTL was determined with qPCR using Cawthon's monochrome multiplex method (Cawthon, 2002, 2009). Briefly, the 2 μl DNA samples were amplified for 40 cycles using either telomere or β-globin primers and the FastStart Universal SYBR Green Master reagent (Roche) in 20 μL final reaction volume. Reactions were run using telomere primers and beta-globin (SCG: single copy gene) primers on ABI 7300 real-time PCR system (Applied Biosystems, CA, United States) according to the following conditions: for telomere 95°C for 10 min, 2 cycles of 95°C for 15 s, 49°C for 15 s and 40 cycles of 95°C for 15 s, 60°C for 15 s, 70°C for 1 min and for β-globin 95°C for 10 min, 40 cycles of 95°C for 15 s, 60°C for 1 min followed by a dissociation (or melt) curve for PCR product verification. The *Ct*-values from both telomere (T) and SCG (S) expression were used to calculate the RTL (T/S ratio) by using the $2^{-(\Delta Ct1-\Delta Ct2)} = 2^{-\Delta\Delta Ct}$ and will be referred as RTL in the following.

Statistics

The RTL values were used in all the statistical testing. The analysis was performed between RTL, and PA intensity levels among the 68-, 69-, and 70-year age groups as well as the four time points (15, 30, 50, current) to assess associations. Subjects were also divided to quartiles based on the PA volume (number of steps) and RTL was compared between quartiles. We used the Kruskall–Wallis test for comparison of continuous variables between groups. For group comparison, including gender and PA frequency and volume quartiles Mann–Whitney *U*-test was used. Correlations between variables (RTL, PA volume, and sedentary time) were assessed using Pearson correlation. Multiple linear regression models were utilized to evaluate the associations of steps with log-transformed RTL. The model was adjusted for age and potential confounder including education (Adler et al., 2013), alcohol consumption (Wang et al., 2017), smoking, BMI (Weischer et al., 2014), triglycerides, high-density lipoprotein (HDL) (Révész et al., 2014), and type 2 diabetes (Salpea et al., 2010). *P*-value of or less than 0.05 was considered significant. Presented numbers are mean ± standard deviation. Statistical analyses were done using IBM SPSS Statistics 21 and SAS 9.3.

RESULTS

Age and Gender

We examined 700 subjects, 296 males and 404 females from the Oulu cohort (**Table 1**). Age was negatively correlated with RTL ($r = -0.185$ and $p = 0.0001$) within the study population and the correlation was stronger in men than in women ($r = -0.210$ and $r = -0.174$, respectively) (**Figure 2**). Since age was significantly associated with telomere length (TL) (**Table 1**), we divided the population into three groups based on age for further analysis.

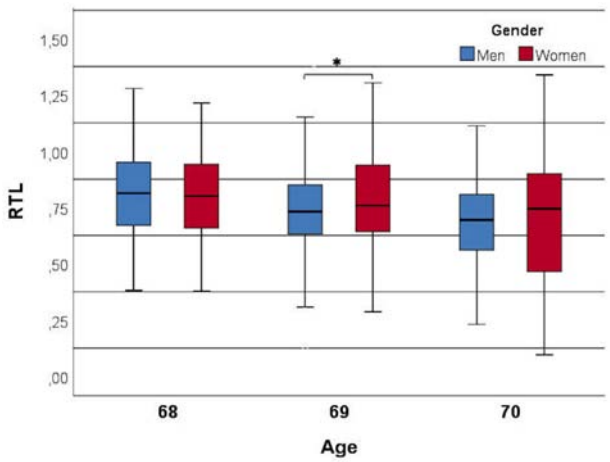

FIGURE 2 | Relationship between relative telomere length (RTL) and age divided by genders. Age is negatively correlated with RTL ($r = -0.185$ and $p = 0.0001$) and a gender difference was observed in the 69-year old subjects ($p = 0.031$). * indicates the significance.

Interestingly, the age-stratified gender difference was observed in the 69-year old group only (N = 397) with women having longer RTL than men (p = 0.037). This difference was not observed in neither the 68- (n = 180) nor the 70-year old group (n = 123; p = 0.678 and p = 0.702, respectively).

Exercise Volume

During the 2-week study period, the subjects took 131799 ± 58535 steps in total and women took significantly more steps than men (women 138479 ± 57557, men 122533 ± 58721, and p = 0.0001) (**Figure 3**), but the positive correlation between total amount of steps and RTL was significant only in men (p = 0.05). The association between steps and RTL in men remained significant after adjustment for age, but not with other confounders (**Table 2**). In the 70-year old group, sedentary time was negatively correlated with RTL (r = −0.247 and p = 0.007), but the total number of steps taken during the 2-week study period was positively correlated with RTL (r = 0.202 and p = 0.04). However, such correlations were not observed with neither the 68- nor the 69-year old group, nor with the whole study population. We also divided the subjects into quartiles using the mean daily steps and compared the RTL between quartiles (**Figure 4**). No significant differences were observed (p-values > 0.05).

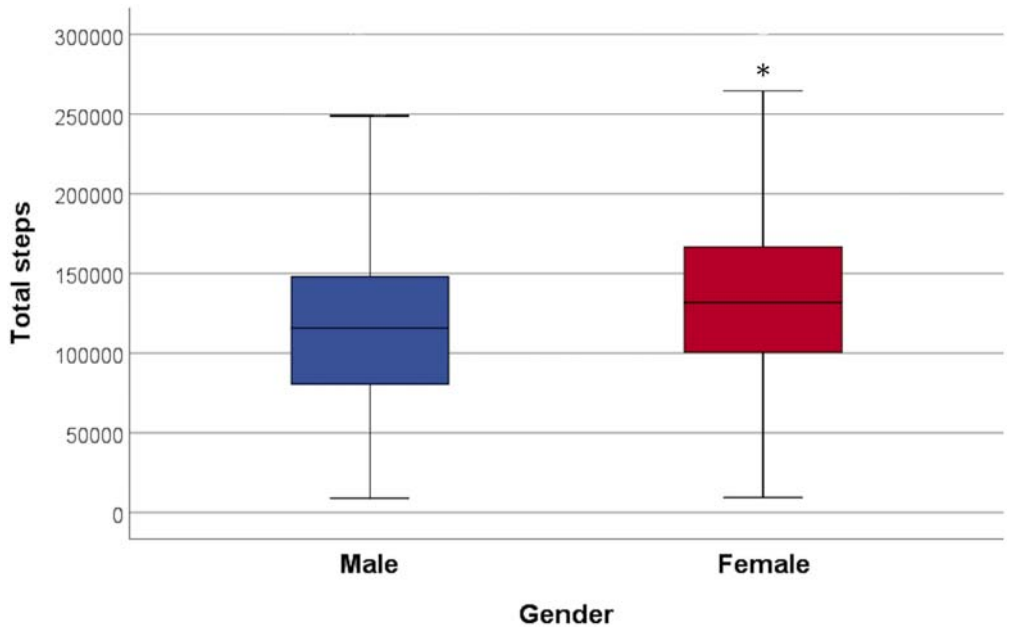

FIGURE 3 | Total steps taken during the 2-week study period in both genders. Females took significantly more steps than males (p = 0.0001). * indicates the significance.

TABLE 2 | Association of mean steps taken and RTL in men (N = 296).

	Model 1			Model 2			Model 3			Model 4		
Variable	**B**	**SE**	***p*-value**	**B**	**SE**	***p*-value**	**B**	**SE**	***p*-value**	**B**	**SE**	***p*-value**
Intercept	−1.034	0.347	0.003	−0.884	0.388	0.024	−1.113	0.501	0.027	−1.113	0.502	0.028
Age	−0.130	0.037	0.000	−0.133	0.040	0.001	−0.131	0.040	0.001	−0.131	0.040	0.001
Mean steps	0.077	0.039	0.049	0.064	0.043	0.143	0.074	0.048	0.126	0.075	0.049	0.124
Education 1				−0.055	0.056	0.330	−0.052	0.057	0.362	−0.052	0.057	0.357
Education 2				0.008	0.049	0.869	0.010	0.050	0.835	0.010	0.050	0.849
Alcohol				−0.006	0.005	0.247	−0.007	0.006	0.222	−0.007	0.006	0.221
Smoker				−0.027	0.060	0.651	−0.021	0.061	0.728	−0.022	0.061	0.716
Triglycerides							−0.011	0.021	0.607	−0.011	0.021	0.602
HDL							0.006	0.059	0.912	0.008	0.059	0.897
BMI							0.005	0.006	0.405	0.004	0.006	0.444
Type 2 diabetes										0.010	0.052	0.841

B = beta estimate, SE = standard error. Model 1 includes RTL, mean steps and age; Model 2 includes Model 1 variables and alcohol consumption, smoking status and education; Model 3 includes variables from Models 1 and 2 and triglycerides, HDL, and BMI. Model 4 includes variables from models 1–3 and type 2 diabetes. Education 1: level of basic education. Education 2: level of professional/vocational education.

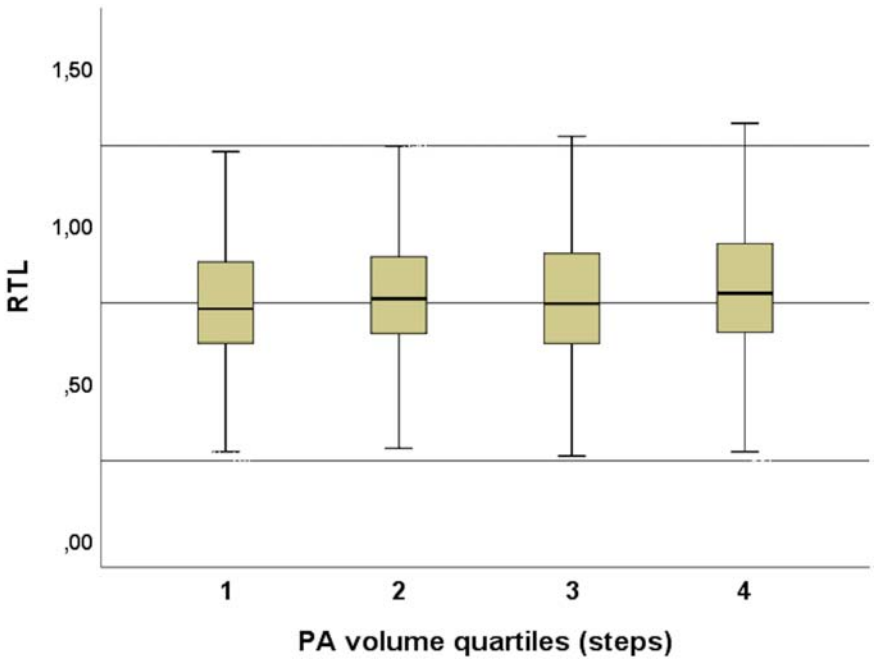

FIGURE 4 | PA volume quartiles and RTL. No significant differences were observed between the quartiles (p-values > 0.05).

Exercise Intensity by Questionnaire

The highest quartile (4th) of MPA (at age 68–70) resulted in significantly different RTL compared to those subjects within the three lowest MPA quartiles (**Figure 5**). The subjects in the highest quartile took significantly more daily steps on average in comparison to those in the other 3 quartiles ($p < 0.001$). This was observed among the 70-year old subjects but not in the 68- or 69-year old age groups. Earlier physical activities at the age of 15, 30, and 50-years old did not reveal any significant differences in current RTL. VPA quartiles at age of 50 and daily average steps at age of 68 to 70 were significantly different ($p = 0.001$).

DISCUSSION

In this study, we investigated the associations of self-reported and objective PA with RTL among elderly adults from the Oulu Birth Cohort 1945. The small age difference in the subjects was negatively correlated with RTL ($r = -0.185$ and $p = 0.0001$) with a stronger correlation in men ($r = -0.210$ and $r = -0.174$, respectively). Telomeres are known to have a high inter-variability, based on epigenetics and telomerase preferences (Nordfjäll et al., 2009). In our cohort, women had higher RTL than men after adjustment for age ($p = 0.037$), as previously described (Weischer et al., 2014). This phenomenon could be explained in part via traditional gender roles still existing in this age group in Finland. For example, men are normally doing the more physically demanding chores (snow shoveling, wood cutting, and renovation) than women who engage more often in everyday tasks such as cleaning and cooking. In addition, women are normally more aware of their health with better nutrition and vitamin supplementation (Radimer et al., 2004; Undén et al., 2008).

Objectively measured PA during a 2-week period with wrist-worn accelerometers was positively correlated with RTL in men at 68–70 years ($r = 0.118$ and $p = 0.05$) and in both genders at 70 years ($r = 0.202$ and $p = 0.04$). The association between steps and RTL in men remained after adjustment for age (model 1) but did not persist after adjustment for other potential confounder (alcohol consumption, smoking, education, triglycerides, HDL, BMI, and type 2 diabetes) (**Table 2**). Women took more steps during the study period, yet the positive association of exercise with RTL was only seen in men. In the 70-year old subjects, sedentary time was negatively correlated with RTL ($r = -0.247$ and $p = 0.007$). Previous studies are in line with our findings. A similar association was observed by Shadyab et al. (2017b) in 1,481 elderly women (aged 79.2 ± 6.7), with a shorter TL in less active subjects (higher sedentary time). In the same subjects, higher amounts of moderate to vigorous PA were associated with longer TL (Shadyab et al., 2017a). In addition, Weischer et al., 2014 observed a significant association between physical inactivity and shorter TL in subjects aged 47 to 76 years ($N = 4,576$ both sexes). These findings suggest that higher levels of physical activity are associated with longer TL (Ludlow and Roth, 2011; Bojesen, 2013). Puterman et al. (2010) studied 63 women (aged 61.9 ± 6.5) and observed that subjects with higher PA levels had less psychological stress (validated stress questionnaire) and longer telomeres. In our cohort, we did not observe an inverted U-shaped relationship between exercise volume and RTL (**Figure 4**), which was reported in other studies involving

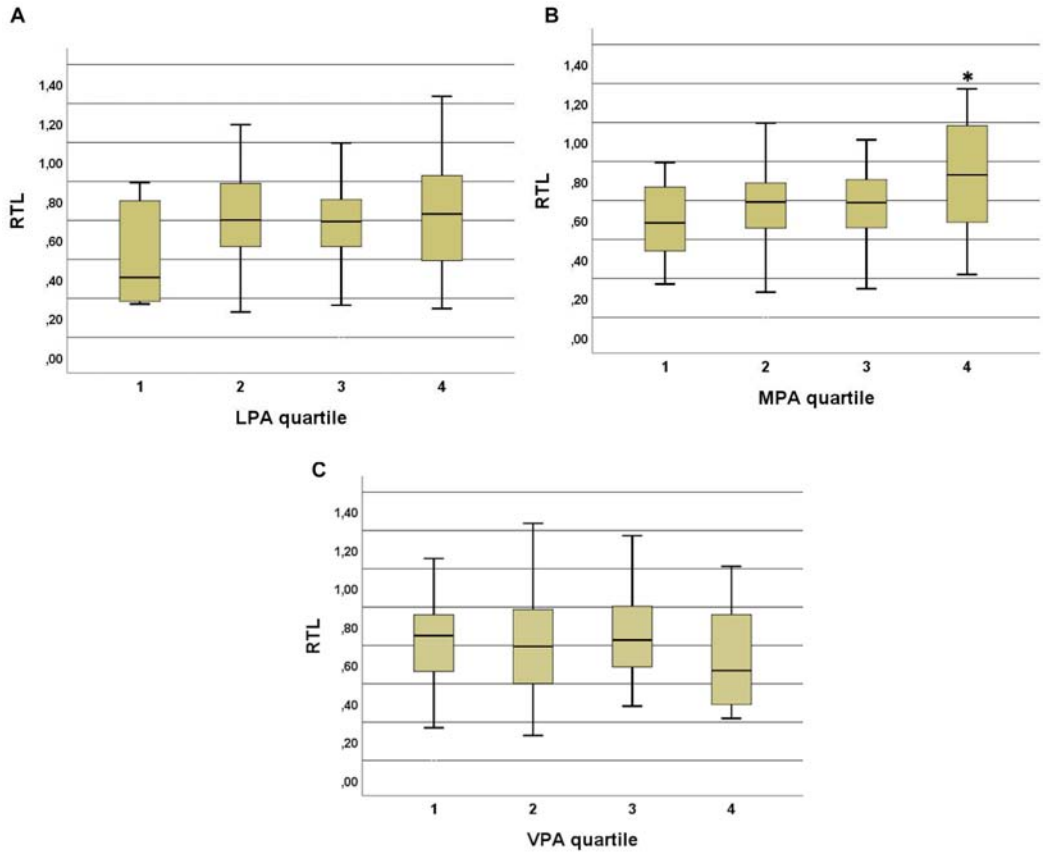

FIGURE 5 | (A) Low intensity physical activity (LPA) quartiles and RTL in 70-year old subjects. No significant correlations were observed between the quartiles but there is a trend to shorter RTL in the quartile with the lowest physical activity (at least 15 min once for a week or less). **(B)** Moderate intensity physical activity (MPA) quartiles and RTL in 70-year old subjects. A significant difference was observed between the highest quartile and three lower quartiles. *p*-values are 0.023 between 4th and 1st, 0.04 between 4th and 2nd, and 0.027 between 4th and 3rd quartiles. People in the highest quartile engage in MPA 5 times or more in a week at least for 15 min at the time. * indicates the significance. **(C)** Vigorous physical activity (VPA) quartiles and RTL in 70-year old subjects. No significant differences were observed between the quartiles. In the highest quartile the RTL is slightly but not significantly lower than in the three other quartiles.

athletes (Kadi et al., 2008; Ludlow et al., 2008; Rae et al., 2010; Mathur et al., 2013). This finding suggests that the relationship seen in athletes might not be applicable to general population and certainly not to the elderly with their usual lack of larger amounts of vigorous exercise. Laine et al. (2015) found no differences between the RTL of former Finnish male athletes and their non-athlete counterparts (N = 599), that were similar in age to our study (athletes 72.7 $\pm$ 6.1 and controls 71.6 $\pm$ 5.6).

We found that only moderate PA was significantly associated with RTL. The 70-year old subjects, who engaged in MPA 5 or more times in week at least 15 min at a time had higher RTL than those subjects, who did less. In accordance with this, subjects in the lowest LPA quartile and highest VPA quartile had lower RTL, but the differences between other quartiles were not significant (**Figure 5**), indicating that MPA is more strongly associated in terms of longer TL in elderly subjects (Ludlow and Roth, 2011). The associations of previous PA at the ages of 15, 30, and 50 with RTL at the age of 70 were not significant. Interestingly, the reported level of VPA at age 50 correlated with the number of steps taken during the measurement period. These data suggest that subjects in this study may have developed PA habits in midlife that continued into old age.

Strengths and Weaknesses of Our Study

This study has several strengths. All participants of our study were born in the same year and lived in the same region. They shared similar conditions in terms of environment, lifestyle and healthcare throughout their lives and have the same ethnic background. Moreover, PA presented in this study was measured objectively. This study also has several limitations. Wrist-worn accelerometers have been shown to overestimate step counts compared to waist-worn accelerometers, which makes it more difficult to compare these results with other studies with different accelerometers (Lee et al., 2015). In contrast, a strong association between self-reported leisure time physical activity and accelerometer-based step counts (Polar Active) in Northern Finland Birth Cohort 1966 study has recently been reported (Niemelä et al., 2019). Furthermore, the questionnaire has been only recently validated at age 46 in a similar study population in

Northern Finland. Our elderly adult participants are considered as "survivors" of that age cohort. Since they were 68–70-years old at the data collection time, we hypothesize that the people with the worst lifestyle and lowest levels of activity did not participate in the study or were deceased. We cannot exclude recall bias, since a self-reported questionnaire was used to obtain data. Further linear regression modeling was not performed since the number of subjects was too small for further multiple linear regression modeling.

CONCLUSION

In conclusion, we found, in an elderly cohort, born in 1945 in Northern Finland (latitude 65° North), that women had longer RTL and performed a higher volume of exercise compared to men. In addition, exercise volume and RTL were correlated positively in men but not in women. Age correlated negatively with RTL even with the age difference of only 2 years. We did not observe an inverted U-shaped relationship between PA volume and RTL. Moderate physical activity at the time of the study was positively associated with RTL.

ETHICS STATEMENT

The study was approved by the ethical committee of the Northern Ostrobothnia Hospital District and has been carried out according to the National legislation and guidelines and the Declaration of Helsinki. Written informed consent was given by all subjects.

AUTHOR CONTRIBUTIONS

K-HH, JL, DG, and SK-K designed the study and provided the funding. VS, KM, and SM did the telomere analysis in the cohort subjects. VS and JJ did the data analysis. VS wrote the first draft of the manuscript. All authors contributed to writing of the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphys.2019.00444/full#supplementary-material

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Exercise and Dietary-Protein as a Countermeasure to Skeletal Muscle Weakness: Liverpool Hope University – Sarcopenia Aging Trial (LHU-SAT)

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Objective: To investigate the effects of a 16-week concurrent exercise regimen [resistance exercise (RE) + functional exercise (FE)] in combination with, or without, a leucine-enriched whey protein isolate supplement on muscle strength, physical functioning, aerobic capacity, and cardiometabolic health in older adults (≥60 years). Physical activity levels were also evaluated 6 months post-cessation of the intervention.

Methods: Forty-six, community-dwelling, previously untrained males, and females [age: 68 ± 5 years (mean ± SD); BMI: 27.8 ± 6.2 kg/m^2] who completed the trial were initially randomized to one of two independent arms [Exercise n = 24 (E); Exercise+Protein n = 22 (EP)]. Both arms completed 16 weeks of RE (performed to fatigue) (2 times/week) with FE (1 time/week) on non-consecutive days. Additionally, EP were administered a leucine-enriched whey protein supplement (3 times/day) for 16 weeks based on individual body-weight (1.5 g/kg/day).

Results: As a result of dietary supplementation, protein intake increased in EP (∼1.2 ± 0.4 to 1.5 ± 0.7 g/kg/day) during the intervention. Maximal strength (1RM) values for leg press (E: +39 ± 7 kg, p = 0.006; EP: +63 ± 7 kg, p < 0.001), chest press (E: +22 ± 4 kg, p < 0.001; EP: +21 ± 6 kg, p < 0.001), and bicep curl (E: +7 ± 0 kg, p = 0.002; EP: +6 ± 1 kg, p = 0.008) significantly increased in E and EP respectively, with no differences between arms (p > 0.05). Physical functioning in the obstacle course (E: −5.1 ± 6.8 s, p < 0.001; EP: −2.8 ± 0.8 s, p < 0.001) and short-physical performance battery scores (E: +0.5 ± 0.5, p = <0.001; EP: +0.4 ± 0.5, p = 0.038), and aerobic capacity in the 6-min walk test (E: +37 ± 24 m, p = 0.014; EP: +36 ± 3 m, p = 0.005) improved in E and EP respectively, with no differences between arms (p > 0.05). No significant change was observed for markers of cardiometabolic health (glycaemic control or blood pressure) (p > 0.05). At follow-up, 86% of older adults reported to performing physical activity ≥1 per week. Of those, 61% were still participating in strength- and cardiovascular- based exercise.

Conclusion: Concurrent exercise (RE + FE) offers a potent method to combat age-related muscle weakness, and our results suggest a high proportion of older adults may continue to exercise unsupervised. However, leucine-enriched whey protein isolate

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supplementation did not confer any additional benefit in those already consuming ample amounts of dietary protein at trial enrolment. Future trials should utilize a whole-foods approach and investigate the effects in frail and non-frail older adults habitually consuming the RDA of protein, to assess if a higher intake of protein is needed to delay the onset of muscle weakness.

Trial Registration: Clinicaltrials.gov Identifier: NCT02912130.

Keywords: aging, muscle weakness, exercise, dietary-protein, leucine

INTRODUCTION

The aging epidemic has led to increased awareness of frailty phenotypes, notably muscle weakness (Fried et al., 2001), which manifests around 50 years of age, and occurs at a 2–5 times more rapid rate than muscle mass loss (Goodpaster et al., 2006). In the United Kingdom alone, estimated annual costs attributed to muscle weakness are £2.5 billion (Pinedo-Villanueva et al., 2018) which emphasizes the urgent need for prevention strategies.

Two prophylactics suggested to curtail muscle weakness are resistance exercise (RE) and dietary-protein. RE is a potent stimulus to increase muscle strength and physical functioning (Fiatarone et al., 1990; Stec et al., 2017) whilst epidemiological data show higher quantities of dietary-protein (>1 g/kg/day) can curb declines in grip strength (McLean et al., 2016) and mobility (Mustafa et al., 2018). Nonetheless, the body of evidence to support the increased requirement of dietary-protein to augment RE effects on muscle strength is inconclusive. Individual trials have failed to show benefits (Verdijk et al., 2009; Leenders et al., 2013; Holwerda et al., 2018) and only when trials are pooled in a meta-analysis does a positive effect appear (Cermak et al., 2012; Morton et al., 2017) although this has not always been the case (Finger et al., 2015; Gade et al., 2018). Disparate findings may be due to total amount, type and timing of supplemented protein, and in particular, sub-optimal intakes of the essential amino acid leucine, the key regulator of muscle anabolism (Devries et al., 2018). Acute trials utilizing isotope tracers have demonstrated an anabolic resistance in older adults, whereby higher dosages of dietary-protein rich in leucine are suggested to overcome this phenomenon (Moore et al., 2015).

Regarding the optimal intensity of RE, similar increases in strength have been evident when comparing moderate and heavy loads in the range of 40–90% of maximum (Morton et al., 2016; da Silva et al., 2018) once total volume is equated for, and lower loads are carried out to fatigue. Nevertheless, as 45.1% of 14,807 older adults (>75 years) suffer chronic musculoskeletal pain (Cimas et al., 2018) refraining from heavy repetitive loading may be a more practical choice to maintain adherence long term. Similar to RE intensity, comparable improvements in strength are apparent with two compared to three weekly sessions in older adults (Silva et al., 2017; Stec et al., 2017).

A Cochrane review (Sherrington et al., 2019) recently highlighted that combining multiple exercises (muscle strengthening, functional, and balance) offset falls in community-dwelling older adults by 34%. Considering this, in addition to the principle of specificity effect (Hawley, 2008; Reilly et al., 2009), there is strong evidence to include RE and functional exercise (FE) in a regimen to obtain the synergistic benefits on muscle strength and physical functioning. In addition, including FE may act as an added stimulus to confer cardiometabolic health benefits on blood pressure, glycaemic control, and aerobic capacity (Whitehurst et al., 2005; Pollock et al., 2018).

With the aforementioned research in mind, the aim of the present two-arm trial [Exercise (E); Exercise+Protein (EP)] was to investigate the synergistic effects of 16-weeks of RE (to fatigue) with FE, in combination with, or without, a leucine-enriched whey protein isolate supplement on muscle strength, physical functioning, and cardiometabolic health in older adults. It was hypothesized EP would demonstrate superior increases in muscle strength (our primary outcome) compared to E. Secondary aims included the effect of treatments on (a) physical functioning, (b) aerobic capacity, and (c) markers of cardiometabolic health which we anticipated to be superior in EP compared to E. Of tertiary interest was to examine physical activity levels 6 months post-cessation of the trial, which we envisaged to be low.

MATERIALS AND METHODS

Subjects

Sample size was based on an average pooled effect size of 0.5 (range 0.1–0.9) from a previous meta-analysis (Cermak et al., 2012), which found greater increases in leg strength with combined RE and dietary-protein vs. RE alone in older adults. Using G*Power (Faul et al., 2007) software and setting power to 80% with alpha at 0.05 (two-tailed) to observe a treatment effect $n \geq 32$ participants were required for final analysis. Recruitment was conducted via online advertisement detailing trial information and enrolment was based on initial telephone screening outlining inclusion and exclusion criteria[1]. To confirm eligibility, participants completed a physical activity readiness questionnaire (PAR-Q) (Thompson et al., 2013) to screen for pre-existing medical conditions. During this time participants were briefed on the nature of the trial, associated risks and benefits before written informed consent was obtained. Participants were excluded with uncontrolled hypertension (160/100 mmHg), hypotension (≤100 mmHg), hyperglycaemia (HbA1c ≥ 10%), on prescribed hormonal and/or anti-inflammatory medication,

[1]https://clinicaltrials.gov/ct2/show/NCT02912130

previous history of scheduled exercise (past 12 months), recent musculoskeletal injury, intolerance to dairy and/or lactose products (for exhaustive list see text footnote 1). For the duration of the trial, participants were instructed to refrain from exercise, and/or nutritional supplements other than administered by the intervention. Ethical approval was sought from the North-West of England NHS Research Ethics Committee United Kingdom (REC No. 16/NW/0480) and the trial was registered at clinicaltrials.gov as NCT02912130.

Trial Design

Following enrolment, forty-six, non-frail, community-dwelling, and previously untrained males and females (aged ≥60–86 years) who completed the trial were initially randomized in a single-blind design to one of two independent arms [Exercise n = 24 (E); Exercise+Protein n = 22 (EP); see **Figure 1**]. All participants attended the clinical laboratories at two separate time points (pre- and post- intervention) where outcome measures were performed. During the intervention, E and EP attended the university sports complex gymnasium thrice weekly for one FE and two RE sessions (supervised by certified exercise trainers) on non-consecutive days for the duration of 16-weeks. EP were administered a leucine-enriched whey protein supplement thrice daily (at breakfast, lunch, and dinner) for 16 weeks based on individual body-weight. Protein supplements were consumed in addition to normal dietary intake. To minimize diurnal variation, the outcome measures were carried out at the same time of day pre- and post- intervention.

Pre- and Post-outcome Measures

Anthropometry

Participants removed shoes, socks, watches, jewelry, and any heavy clothing prior to height (nearest 0.1 m; SECA 213 Stadiometer) and weight (nearest 0.1 kg; TANITA MC-180MA) measurements. Body mass index (BMI) was calculated from the above measurements using the following validated equation: body-weight (kg)/height (m^2).

Muscle Strength

Strength was evaluated via 5-repetition maximum (RM) using established guidelines (Baechle and Earle, 2008) on the following exercises in orderly fashion: leg press, chest press, and

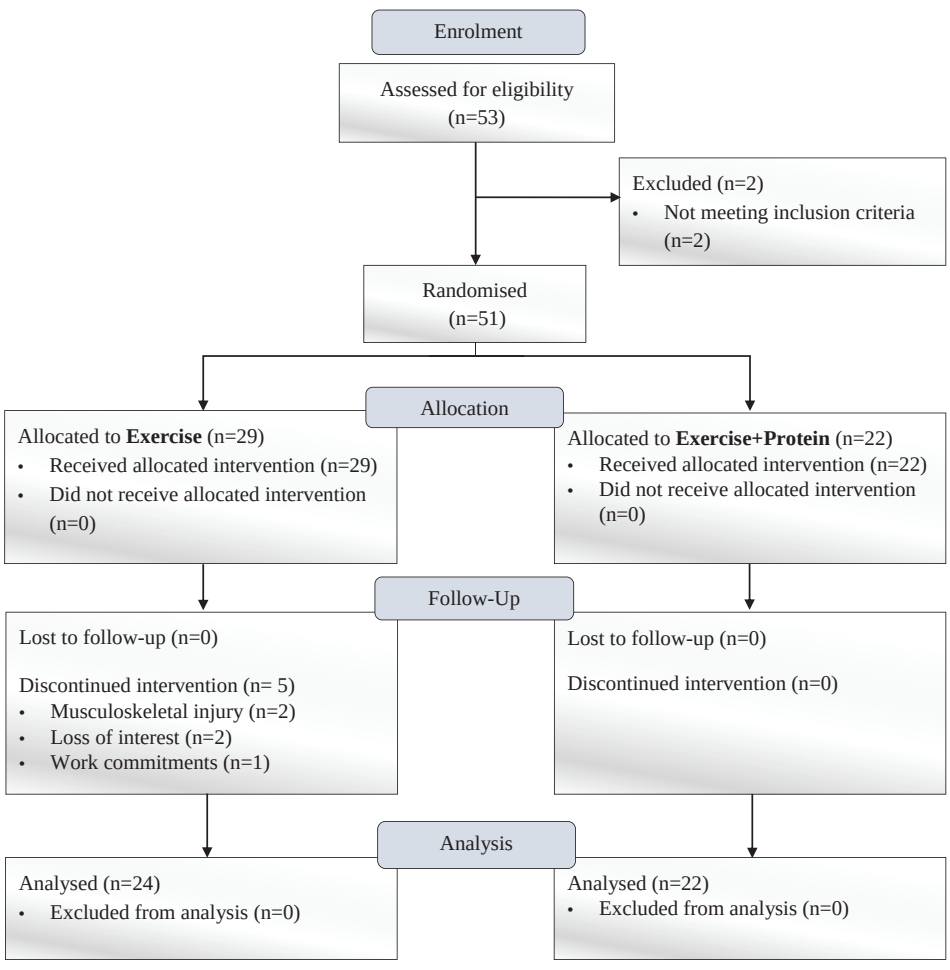

FIGURE 1 | Flow chart of experimental trial.

bicep curl. Testing was performed on resistance machines (leg press and chest press) and with a barbell (bicep curl) using Technogym equipment at the university sports complex gymnasium. A ∼5 min low-intensity cardiovascular warm up was first conducted on either a motorized treadmill, cross-trainer, or bike. Lifting began with a self-selected moderate weight for 15 repetitions followed by 2 min rest before participants completed a further 10 repetitions with an increased weight selected by the exercise trainer. If full range of motion with correct posture was achieved the load was increased by 5 kg and 10 kg for upper- and lower- body, respectively. This process was continued with 2 min breaks until the true 5RM was obtained. 5RM values were then transformed to 1RM values using the previously validated equation (Brzycki, 1993) for strength testing in older adults (Wood et al., 2002). Final 1RM values in kilograms (kg) were used for analysis.

Physical Functioning and Aerobic Capacity

Standardized operating procedures were followed for the short-physical performance battery (SPPB) (Guralnik et al., 1994) which consisted of three timed components: standing balance, 4-m gait speed, and time to complete five chair-stands. Participant scores for each component were totalled between 0 and 12 used for analysis. The obstacle course was re-adapted from Steele et al. (2017) and consisted of a 25 m marked course incorporating 90 and 180 degree turns (**Figure 2**). Using a stopwatch to record time, participants were instructed to rise from the floor and carry a kettlebell weight (10 kg for males, 5 kg for females) as fast as possible around the course. The stopwatch stopped once the participant was re-seated on the floor at the finish line. Time in seconds (s) was used for analysis. For the 6 min walk test (6MWT) standardized operating procedures were followed (ATS Committee on Proficiency Standards for Clinical Pulmonary Function Laboratories et al., 2002). A 30 m track was marked in an environmentally controlled laboratory (17°C) with chairs placed at both ends. Participants were instructed to walk up and down the track covering as much ground as possible within 6 min. Participants were reminded the test was self-paced and if needed a rest was permitted; however, the stopwatch would continue to run. Participants completed two 6MWT with a 10 min break between tests, Average of the two distances in meters (m) was used for analysis.

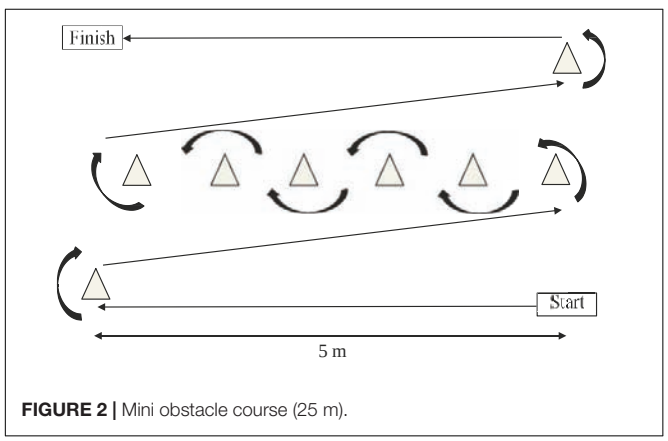

FIGURE 2 | Mini obstacle course (25 m).

Blood Pressure and Fasting Blood Samples

Participants were laying rested on a medical bed for ∼5 min prior to blood pressure measurement. An inflatable cuff (SphygmoCor® CPV system; ScanMed Medical) was applied to the upper arm directly over the brachial artery and subsequent systolic and diastolic blood pressure readings (mmHg) were taken and used for analysis. A 35 μm capillary fingerstick blood sample was then collected in sterile conditions for the subsequent determination of plasma glucose (mmol/L; Alere Cholestech LDX Analyzer, Chesire, United Kingdom) and glycated hemoglobin (HbA1c %; Alere, Afinion^{TM}, AS100, Cheshire, United Kingdom).

Physical Activity Follow-Up Survey

Six months post-cessation of the intervention all participants were re-contacted and asked to fill out an online survey (designed via Bristol Online Survey[2]). Survey was re-adapted from Forkan et al. (2006) and consisted of three multiple-choice questions. (1) How many times have you exercised in the past 4 weeks? (2) If you exercised in the past 4 weeks what type of exercise was it? (3) How long did each session last? Individual responses were totalled and analyzed to illustrate a %.

Exercise Intervention

Participants completed a gym induction and attended a familiarization day where the correct range of motion for each RE exercise was demonstrated to ensure technique and minimize injury risk. Participants also practiced lifting the weight to fatigue (defined as the point where the weight could no longer be lifted with correct posture). Participants were provided with a booklet detailing weekly sessions, specific exercises, and shown how to track weights. For FE, participants were shown the correct movement for each exercise and familiarized with the Borg scale during a practice session. Session attendance was recorded on arrival at the gymnasium reception desk. Average attendance was totalled to give a %.

Resistance Exercise

Each sessions lasted ∼50 min; with 5 min warm up of low-intensity exercise on either a motorized treadmill, cross-trainer or bike, then continued with 45 min of whole-body REs. Participants first completed one upper- and lower- body warm up with a lightweight. Participants then self-selected a moderate weight and completed 2 sets to fatigue separated by 3 min/between sets and 3 min/between exercises on each of the following machines in orderly fashion: leg press, chest press, calf press, shoulder press, seated row, and back extension. Bicep curl was performed last using a free weighted barbell due to no machine-based option. Weight was increased for upper- and lower- body exercises by 2.5 and 5 kg, respectively, once the participant completed ≥12 repetitions in both working sets. Maximal effort and progressive overload was encouraged by the exercise trainer.

[2]https://admin.onlinesurveys.ac.uk

Functional Exercise

Warm-up began with ∼10 min of low-intensity dancing to participants preferred choice of music. FE session consisted of 12 stations re-adapted from Whitehurst et al. (2005) with 1 min of exercise performed at each individual station before moving in order to the next. Each station was marked with the exercise station name, assigned a station number (between 1 and 12) and marked with a visible Borg CR-10 scale effort sheet. Participants completed the FE circuit 3 times with 3 min breaks between sets (see **Figure 3**). Participants were instructed to provide high effort throughout the session demonstrating a level of 7–10 on the Borg scale (Borg and Kaijser, 2006).

Protein Supplementation and Dietary Control

All participants recorded their energy intake via 4-day food diaries pre- and post- intervention. Instructions were given how to correctly weigh food, measure liquids, and fill in the diaries. Protein supplements were weighed on scales (Weighstation Electronic Platform Scale, Devon, United Kingdom) and sealed in sachet bags (Tesco Stores, United Kingdom) according to participants' individual body-mass (g/kg/body-weight). Participants in EP were administered a Vanilla flavored Whey Isolate Protein supplement (MyProtein, Northwich, Cheshire, United Kingdom) (at: 1.5 g/kg/day; 0.5 g/kg/meal) enriched with Leucine (MyProtein, Northwich, United Kingdom) (at: 0.09 g/kg/day; 0.03 g/kg/meal) and mixed with 200 ml of water which was ingested thrice daily (breakfast, lunch, dinner) for 16-weeks. This dosage has previously shown to overcome the anabolic resistance among older adults (Moore et al., 2015).

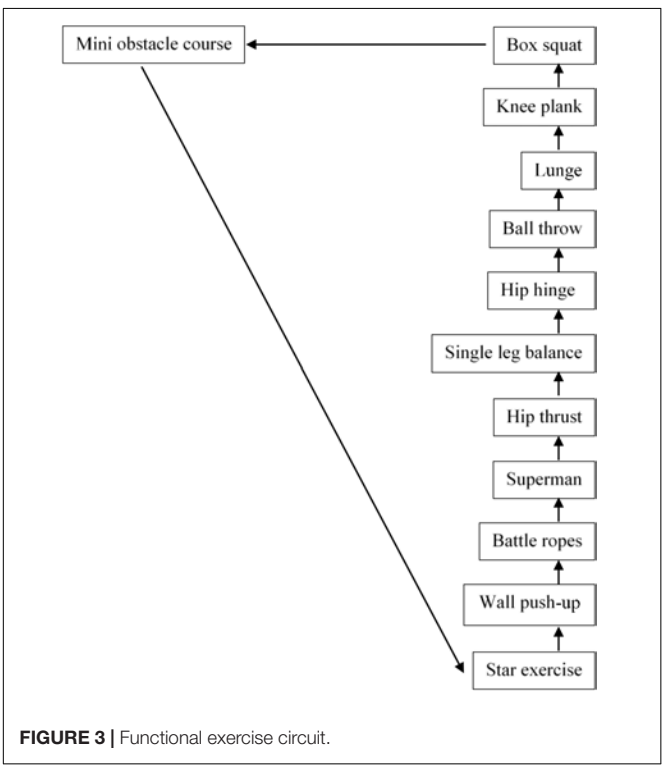

FIGURE 3 | Functional exercise circuit.

Participants were reminded the protein supplement was to be consumed in addition to normal dietary intake. Adherence was assessed via self-report supplement logs and by counting returned sachets. Compliance with the protein supplement was totalled across the intervention to show a %.

Statistical Analysis

Statistical analysis was performed using SPSS Statistics 24 (IBM Corporation, New York, United States). Food diaries were analyzed for energy and protein content through dietary analysis software (Nutritics LTD., Ireland). All data were checked for normality via Shapiro-Wilk test, which were violated for muscle strength and physical function measures. Percentage change and log transformations were unsuccessful at normalizing the data therefore non-parametric methods were utilized. Within-arm time effects (pre- and post- intervention) were analyzed by Wilcoxon-ranked paired tests. Between-arm differences (E vs. EP) were analyzed by Kruskal-Wallis (H) tests. Normality tests showed normal distribution for anthropometry, blood pressure, blood glucose, glycated hemoglobin, and food diary measures therefore parametric testing was utilized. Baseline comparisons were analyzed by students unpaired (t) tests. Independent arms were analyzed using a mixed model ANOVA with two arm levels (E vs. EP) and two time levels (pre- and post- intervention). If between arm effects were present they were followed up using Bonferroni *post hoc* comparisons. Mauchly's test of sphericity was used to check homogeneity of variance; where necessary, any violations of the assumption were corrected using the Greenhouse–Geisser adjustment. Data are expressed as mean (±) standard deviation throughout. For descriptive purposes, percentage (±) is calculated from mean values. The alpha level for statistical significance was set at $p < 0.05$ *a priori*.

RESULTS

Subjects

Participants included in the final analysis were distributed similarly in each arm and when split by gender no difference was detected ($p = 0.55$). Additionally, arms did not differ in any baseline measure ($p > 0.05$) (see **Table 1**).

Exercise and Dietary Adherence

Participants in E and EP attended 77 ± 10% and 78 ± 10% of their prescribed exercise sessions, respectively. A lower degree of compliance was observed with dietary- protein supplementation: EP = 43 ± 14%. As a result of supplementation, protein intake increased from ∼1.2 ± 0.4 at baseline to 1.5 ± 0.7g/kg/day in EP during the intervention period.

Effect of Intervention

Anthropometry, Blood Pressure, and Blood Measures

No within- or between- arm differences were observed for height, weight, BMI, blood pressure, plasma glucose or glycated hemoglobin ($p > 0.05$) (**Table 2**). Although minor (non-significant) decreases in systolic blood pressure (E: 142 ± 19

TABLE 1 | Baseline characteristics of participants.

Parameter	E	EP	*p* value
n = [number]	24	22	
Gender [male/female]	12/12	9/13	0.55
Age [years]	66 ± 4	69 ± 6	0.16
Height [m]	1.68 ± 0.1	1.64 ± 0.1	0.13
Weight [kg]	79.5 ± 21.6	74.2 ± 18.1	0.32
BMI [kg/m^2]	28.1 ± 7.4	27.4 ± 4.9	0.63
Plasma glucose [mmol/L]	5.5 ± 0.6	5.4 ± 0.8	0.90
HbA1c [%]	5.5 ± 0.3	5.4 ± 0.3	0.67
Systolic blood pressure [mmHg]	142 ± 19	147 ± 17	0.36
Diastolic blood pressure [mmHg]	83 ± 16	82 ± 9	0.81
Leg press 1RM [kg]	131 ± 15	100 ± 48	0.06
Chest press 1RM [kg]	36 ± 16	36 ± 15	0.70
Bicep curl 1RM [kg]	19 ± 7	20 ± 6	0.58
SPPB [0–12]	11.5 ± 0.7	11.6 ± 0.7	0.31
Obstacle course time [s]	24.6 ± 12.3	22.0 ± 3.6	0.58
6MWT [m]	579 ± 83	582 ± 67	0.84
Energy intake [kcal/d]	1810.5 ± 385.7	1728.1 ± 359.5	0.55
Protein intake [kcal/d]	81.50 ± 27.1	77.26 ± 21.9	0.65
Protein intake [g/kg/day]	1.10 ± 0.4	1.16 ± 0.4	0.68
Protein intake [% total energy]	18 ± 4	18 ± 3	0.96
Total carbohydrate intake [g/day]	191.81 ± 40.2	168.8 ± 41.5	0.14
Total carbohydrate intake [% total energy]	43 ± 6	39 ± 6	0.11
Total fat intake [g/day]	69.75 ± 18.4	69.56 ± 23.1	0.98
Total fat intake [% total energy]	35 ± 6	36 ± 7	0.70

Data are shown as means ± standard deviations. No significant differences were detected between baseline treatments (E, exercise; EP, exercise + protein) (p > 0.05). HbA1c, glycated hemoglobin; 1RM, 1 repetition maximum; SPPB, short physical performance battery; 6MWT, 6-min walk test.

to 137 ± 13, −5 mmHg; EP: 147 ± 17 to 143 ± 17, −4 mmHg) were evident from pre- to post-intervention in E and EP, respectively.

Muscle Strength

Following 16 weeks of progressive resistance and FE 1RM values for leg press (E: 131 ± 58 to 170 ± 51 kg, +30%, $p = 0.006$; EP: 100 ± 48 to 163 ± 55 kg,+63%, $p < 0.001$), chest press (E: 36 ± 16 to 58 ± 20 kg, +60%, $p < 0.001$; EP: 36 ± 15 to 57 ± 21 kg, +58%, $p < 0.001$) and bicep curl (E: 19 ± 7 to 26 ± 7 kg,+37%, $p = 0.002$; EP: 20 ± 6 to 26 ± 7 kg, +30%, $p = 0.008$) significantly increased from pre- to post-intervention in E and EP, respectively. However, no between-arm differences were observed ($p > 0.05$; **Figure 4**).

Physical Functioning and Aerobic Capacity

Time to complete the obstacle course (E: 24.6 ± 12.3 to 19.5 ± 5.5 s, +21%, $p < 0.001$; EP: 22.0 ± 3.6 to 19.2 ± 4.1 s, +13%, $p = p < 0.001$), performance in the SPPB (E: 11.5 ± 0.7 to 12.0 ± 0.2points, +4%, $p = <0.001$; EP: 11.6 ± 0.7 to 12.0 ± 0.2 points, +3%, $p = 0.038$) and aerobic capacity in 6MWT (E: 579 ± 83 to 616 ± 107 m, +6%, $p = 0.014$; EP: 582 ± 67 to 618 ± 64 m, +6%, $p = 0.005$) significantly improved from pre- to post-intervention in E and EP, respectively. No between-arm differences were observed ($p > 0.05$; **Table 3**).

Physical Activity Levels: Post-trial Follow-Up

Forty-two out of 46 participants completed the 6-months post-trial physical activity survey. No significant differences were observed between arms for any survey question ($p > 0.05$). Pooled results showed 86% (36/42) were still exercising at least 1/week with 14% (6/42) not exercising. Of those subjects still exercising 25% (9/36) reported to performing aerobic exercise (cardiovascular based, i.e., walking, cycling, jogging, swimming, and yoga), 14% (5/36) reported performing RE (weight-bearing, i.e., lifting weights, body-weight exercises) and 61% (22/36) reported to performing both. The duration of these exercise sessions varied between 45 min (33%) (12/36), 60 min (33%) (12/36), and >60 min (33%) (12/36) (see **Figure 5**).

DISCUSSION

We report 16 weeks of progressive resistance and FE (3 times/week) significantly improved muscle strength, physical

TABLE 2 | Effect of intervention on anthropometry, blood pressure, and blood measures.

Parameter	E			EP			
	Pre	Post	Time *p*	Pre	Post	Time *p*	Time*group
Height [m]	1.68 ± 0.1	1.68 ± 0.1	1.000	1.64 ± 0.1	1.64 ± 0.1	1.000	0.302
Weight [kg]	79.5 ± 21.6	78.7 ± 19.8	0.309	74.2 ± 18.1	73.6 ± 17.5	0.970	0.374
BMI [kg/m^2]	28.1 ± 7.4	27.8 ± 6.6	0.319	27.4 ± 4.9	27.3 ± 4.5	0.977	0.379
Plasma glucose [mmol/L]	5.5 ± 0.6	5.5 ± 0.8	0.852	5.4 ± 0.8	5.4 ± 0.8	0.516	0.576
HbA1c [%]	5.5 ± 0.3	5.5 ± 0.4	0.339	5.4 ± 0.3	5.5 ± 0.3	0.378	0.821
Systolic pressure [mmHg]	142 ± 19	137 ± 13	0.258	147 ± 17	143 ± 17	0.329	0.894
Diastolic pressure [mmHg]	83 ± 16	82 ± 8	0.413	82 ± 9	83 ± 9	0.810	0.414

Values are means ± standard deviations. No significant differences between treatments (E, exercise; EP, exercise + protein) (p > 0.05). HbA1c, glycated hemoglobin.

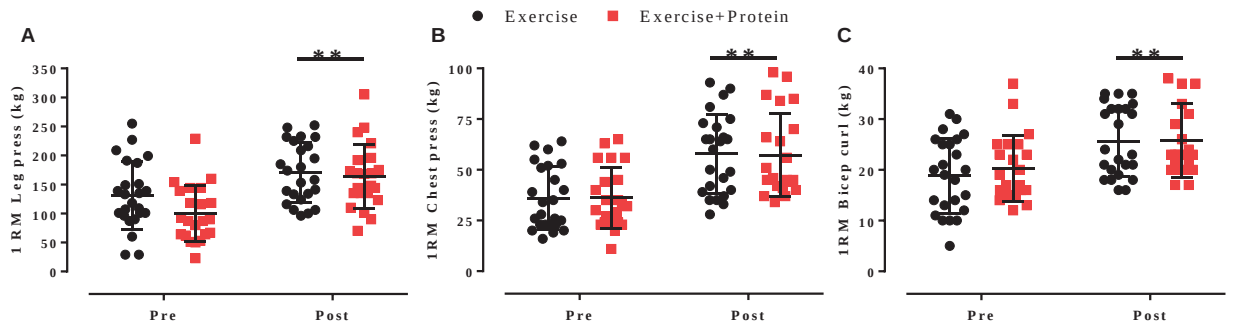

FIGURE 4 | 1RM values for **(A)** leg press, **(B)** chest press, and **(C)** bicep curl in response to independent treatments (E, black; EP, red). Individual data points are shown with horizontal line indicating the mean and error bars representing the standard deviation. Within-arm time effects were evident post-intervention for leg press, chest press, and bicep curl all (*$p < 0.05$). No between-arm effects observed ($p > 0.05$).

TABLE 3 | Effect of intervention on physical function and aerobic capacity.

Parameter	E			EP			
	Pre	Post	Time *p*	Pre	Post	Time *p*	Time*group
SPPB [1–12]	11.5 ± 0.7	12.0 ± 0.2	<0.001	11.6 ± 0.7	12.0 ± 0.2	0.038	0.924
Obstacle course time [s]	24.6 ± 12.3	19.5 ± 5.5	<0.001	22.0 ± 3.6	19.2 ± 4.1	<0.001	0.930
6MWT [m]	579 ± 83	616 ± 107	0.014	582 ± 67	618 ± 64	0.005	0.974

Values are means ± standard deviations. No significant differences between treatments (E, exercise; EP, exercise + protein) ($p > 0.05$). SPPB, short physical performance battery; 6MWT, 6-min walk test.

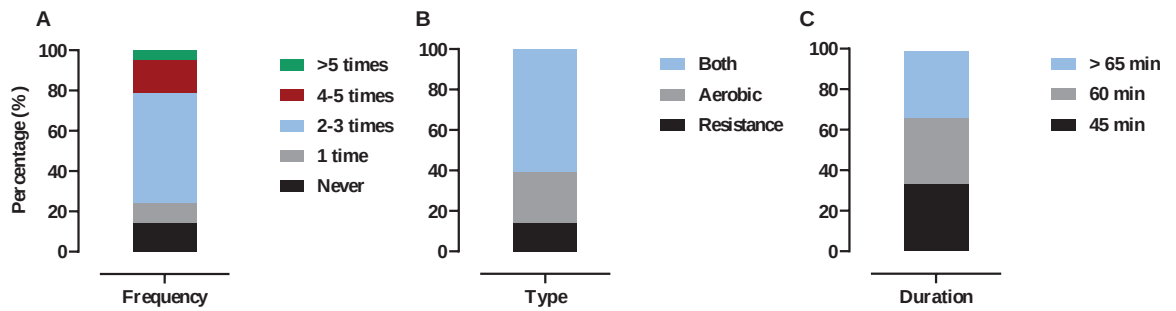

FIGURE 5 | Physical activity levels 6 months post-trial. Values are presented as pooled responses (%) from survey questions. **(A)** How many times have you exercised in the past 4-weeks? **(B)** If you exercised in the past 4-weeks what type of exercise was it? **(C)** If you exercised in the past 4-weeks how long did each session last?

TABLE 4 | Dietary-intake from self-recorded 4-day food diaries.

Parameter	E			EP			
	Pre	Post	Time *p*	Pre	Post	Time *p*	Time*group
Energy intake [kcal/d]	1810.5 ± 385.7	1944.1 ± 568	0.282	1728.1 ± 359.5	1969.3 ± 429.9	0.012	0.454
Protein intake [kcal/d]	81.50 ± 27.1	77.63 ± 20.5	0.512	77.26 ± 21.9	109.61 ± 30.8*	<0.001	0.002
Protein intake [g/kg/body mass/day]	1.10 ± 0.4	1.04 ± 0.3	0.361	1.16 ± 0.4	1.63 ± 0.5*	<0.001	<0.001
Protein intake [% total energy]	18 ± 4	16 ± 3	0.193	18 ± 3	23 ± 6*	0.004	0.003
Total carbohydrate intake [g/day]	191.81 ± 40.2	211.09 ± 68.3	0.674	168.8 ± 41.5	187.8 ± 59.7	0.202	0.989
Total carbohydrate intake [% total energy]	43 ± 6	44 ± 6	0.760	39 ± 6	38 ± 7	0.292	0.398
Total fat intake [g/day]	69.75 ± 18.4	72.96 ± 21.7	0.174	69.56 ± 23.1	75.49 ± 24.4	0.165	0.744
Total fat intake [% total energy]	35 ± 6	34 ± 5	0.719	36 ± 7	34 ± 7	0.396	0.688

*Values are means ± standard deviations. *Indicates between-arm difference post-intervention ($p < 0.05$).*

functioning, and aerobic capacity without influencing blood pressure or glycaemic control in previously untrained older adults. In addition, leucine enriched-whey protein supplementation (3 times/day) did not confer any additional benefit on these outcomes.

We primarily sought to investigate if leucine enriched-whey protein supplementation would augment muscle strength during combined exercise training in older adults. Following recommendations (Paddon-Jones and Rasmussen, 2009; Bauer et al., 2013) we provided ample amounts of dietary-protein (0.5 g/kg/meal) enriched with leucine (0.03 g/kg/meal; >3 g per serving) thrice daily to maximize the muscle protein synthetic response (Moore et al., 2015). Despite substantial increases in muscle strength (**Figure 4**) and physical/aerobic performance (**Table 3**) we observed no difference between treatments. This finding is in line with existing data (Kukuljan et al., 2009; Verdijk et al., 2009; Leenders et al., 2013; Stragier et al., 2016; Holwerda et al., 2018) which failed to show a synergistic effect of RE and dietary-protein in strength among community-dwelling older adults. Similar to the above trials, our population of older adults were non-frail i.e., demonstrated high baseline SPPB (11.5 ± 0.7) and 6MWT (583 ± 75) scores. In contrast, benefits have been observed in pre-frail/functionally impaired older adults with lower habitual levels of dietary-protein (Cawood et al., 2012; Tieland et al., 2012). Thus, the relative good health of our population who were habitually consuming adequate amounts of dietary-protein (∼1.2 ± 0.4 g/kg/day) may have masked any effect of supplementation (**Table 4**). Despite increasing dietary-protein intake from ∼1.2 ± 0.4 to 1.5 ± 0.7 g/kg/day during the present trial, adherence (43 ± 14%) was considerably lower than others (Verdijk et al., 2009; Bell et al., 2017) although similar in those attempting to supplement 3 times/day (Norton et al., 2016). Considering this, coupled with the undesirable verbal feedback relating to supplement taste we recommend future trials use a whole-food approach to increase palatability and adherence as previously described (Haub et al., 2002; Wright et al., 2018).

All strength measures improved from pre- to post-intervention by >30% (**Figure 4**) adding to the current body of research (Charette et al., 1991; Latham et al., 2004; Nilwik et al., 2013; Bell et al., 2017) demonstrating prolonged resistive exercise modalities (≥12 weeks) are a potent method to combat age-related muscle weakness. Together, these data offer an alternative approach for older adults who may be reluctant to use heavy loads due to health or personal constraints.

The observed increases in strength were accompanied by a favorable shift in physical functioning and aerobic capacity (**Table 3**). Whilst difficult to distinguish which part of the multifaceted exercise regimen contributed specifically to these improvements, each may have played a complementary role. For instance, RE increases in strength can improve SPPB performance (Tieland et al., 2012, 2015) whereas FE may have predominately enhanced mobility on the obstacle course (Rosendahl et al., 2008) and provided that added stimulus to increase endurance on the 6MWT (Whitehurst et al., 2005). In support, three studies (Arnarson et al., 2013; Kawada et al., 2013; Oesen et al., 2015) found no effect of RE on 6MWT distance, whilst in the present trial and in others (Bell et al., 2017) combining RE with endurance elements of training resulted in improved 6MWT distance. It is difficult to elaborate further as it was not the purpose of the trial to compare these exercise modalities, and associations between neuromuscular attributes and performance indices are not fully understood (Jacob et al., 2018). Nonetheless, the above findings are clinically relevant considering muscle strength declines at an annual rate of ∼2–3% after the fifth decade of life (Goodpaster et al., 2006) and is adversely characterized by reductions in functional capacity (Pavasini et al., 2016), and activities of daily living (Rantanen et al., 2002).

Our multifaceted exercise regimen was designed to optimize muscle strength, physical functioning, aerobic capacity and metabolic health all of which deteriorate with age (Pendergast et al., 1993; Niccoli and Partridge, 2012). Regarding the latter, we failed to observe a change in markers of cardiometabolic health (**Table 2**) which is in contrast to others (Bell et al., 2017) employing combined strength and high-intensity interval exercise. Thus, we postulate the lack of adaptation in glycaemic control/blood pressure may be due to an insufficient intensity of the exercise regimen employed, or alternatively, due to a lack of reduction in body-weight which may have concealed alterations.

Exercise adherence was high (78 ± 10%) across the 16-week intervention period and was even higher during follow-up (6 months post-intervention) with 86% (36/46) of previously untrained older adults reporting to performing physical activity ≥1 per week (**Figure 5**). Of those, 61% (22/36) were participating in strength- and cardiovascular- based exercise which aligns with current exercise recommendations for older adults (Nelson et al., 2007). The above figures are promising considering older adults are highlighted as the least active section of society with astonishingly low numbers (<5%) meeting guidelines (Davis et al., 2011; Loustalot et al., 2013; Sun et al., 2013; Van Holle et al., 2014; Dalbo et al., 2015). By continuing to perform concurrent exercise our older adults are inevitably reducing the risk of age-related disease (Vellas et al., 2018) and mortality (García-Hermoso et al., 2018). Even slight increases in RE participation rates (as achieved here) may significantly relieve the economic burden of aging as costs attributed to muscle weakness are estimated at an annual £2,707 per person in the United Kingdom alone (Pinedo-Villanueva et al., 2018).

Limitations

A clear drawback of our trial was the lack of compliance (43 ± 14%) to dietary-protein supplementation. As mentioned, future research should use a whole-food approach as greater adherence rates (>90%) have been evident (Haub et al., 2002; Wright et al., 2018). Another perceived limitation may relate to our population of older adults who were non-frail. By incorporating frail older adults, perhaps greater effects of treatments may have been observed. However, as mounting commentary (Paddon-Jones and Rasmussen, 2009; Bauer et al., 2013) advocate higher dietary-protein intakes (≥1.2 g/kg/day) for older adults it would be unwise to examine the effects in functionally impaired populations alone. For public health mandates to endorse a greater intake of dietary-protein above

the current RDA (0.8 g/kg/day); evidence needs to be established across various populations (i.e., in community-dwelling and institutionalized older adults).

CONCLUSION

To conclude, 16 weeks of progressive resistance and FE (3 times/week) significantly improved muscle strength, physical functioning and aerobic capacity without affecting blood pressure or glycaemic control in previously untrained older adults. In addition, leucine-enriched whey protein supplementation (3 times/day) did not yield further benefits. Nonetheless, 86% (42/46) of older adults were still performing strength- and cardiovascular- based exercise 6-months post-trial demonstrating clinical relevance. Finally, future research should focus on methods to incorporate high dietary-protein intakes (∼1.5 g/kg/day) through naturally occurring food sources in frail and non-frail older adults habitually consuming the RDA of protein. In turn, this may improve adherence rates and enable the efficacy of combined RE with dietary-protein on muscle strength to be evaluated.

AUTHOR CONTRIBUTIONS

BK, KM, FA, and OK have made substantial contributions to the trial design, data collection and interpretation, and are fully conversant with its content. BK wrote the full manuscript.

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Effect of Aerobic Exercise on Inflammatory Markers in Healthy Middle-Aged and Older Adults: A Systematic Review and Meta-Analysis of Randomized Controlled Trials

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Background: Chronic inflammation plays a significant role in accelerating the aging process and is closely associated with the initiation and progression of a broad range of age-related diseases. Physical exercise is considered beneficial in alleviating these conditions, but the effects of aerobic exercise on inflammatory markers in a healthy population should be furtherly clarified.

Objective: The purpose of this systematic review and meta-analysis was to evaluate the effect of aerobic exercise on inflammatory markers in middle-aged and older adults.

Methods: The literature search was conducted utilizing PubMed, Web of Science, Embase, and the Cochrane Library from their inception through April 2018, and the reference lists were screened to identify appropriate studies. Only randomized controlled trials that investigated the effect of aerobic exercise on inflammatory markers in middle-aged and older adults were eligible for this review.

Results: Eleven studies involving 1,250 participants were retrieved from the databases for analysis. The pooled results showed that aerobic exercise significantly reduced inflammatory markers (C-reactive protein (CRP): SMD = 0.53, 95% CI 0.26–0.11, $p = 0.0002$; tumor necrosis factor-alpha (TNF-α): SMD = 0.75, 95% CI 0.31–1.19, $p = 0.0007$; interleukin 6 (IL-6): SMD = 0.75, 95% CI 0.31–1.19, $p = 0.0007$). No significant improvement was found in relation to interleukin 4 (IL-4).

Conclusions: Aerobic exercise may have a positive effect on reduction of CRP, TNF-α, and IL-6 in middle-aged and older adults. Further randomized controlled trials (RCTs) need to be conducted to determine the effect of aerobic exercise on additional inflammatory markers in the population of middle-aged and older adults.

Keywords: aerobic exercise, inflammatory markers, CRP, TNF-α, IL-6, IL-4

INTRODUCTION

Aging is a complex process that is compounded by a combination of environmental, genetic, and epigenetic factors. Chronic inflammation plays an increasingly significant role in health status by accelerating the aging process (Franceschi and Campisi, 2014). Low-grade, persistent chronic inflammation occurs in the majority of the middle-aged and elderly population and is thought to be an accelerator of biological aging (Fougère et al., 2016). An increasing number of studies have demonstrated that chronic inflammation is closely associated with the initiation and progression of a broad range of age-related diseases, such as cardiovascular disease, cancer, diabetes, Alzheimer's disease, and other neurodegenerative diseases and is an independent risk factor for mortality in healthy adults (Kalogeropoulos et al., 2010; Argilés et al., 2015; Bonaccio et al., 2016; Landman et al., 2016; Uchoa et al., 2016; Korniluk et al., 2017; Lang et al., 2018). Moreover, there is strong evidence that the development of age-related diseases is linked to low-grade elevation of circulating inflammatory mediators (Singh and Newman, 2011). Therefore, future interventional researches should focus on preserving overall homeostatic balance and controlling inflammatory status in the aging patient.

Physical exercise is well-recognized as an important strategy for reducing the risk of age-associated diseases (Sparling et al., 2015), and recent research has focused on the role of exercise in the improvement of the inflammatory profile. Large population-based cross-sectional and cohort studies consistently show an inverse association between markers of systemic inflammation and physical exercise; lower inflammatory biomarker concentration is observed in subjects with more frequent and more intense physical exercise (Colbert et al., 2004; Beavers et al., 2010; Lee et al., 2012; Streese et al., 2018). However, data from interventional studies designed to definitively examine the effects of physical exercise on inflammation are limited, and results are inconclusive. For example, in Woods' review of randomized clinical trials, there was evidence indicating that regular exercise could induce loss of fat mass and adipose tissue, which is known to contribute to systemic inflammation (Woods et al., 2012). Another meta-analysis of eight RCTs also showed that exercise could reduce inflammatory markers in older adults, thereby decreasing the risk of developing age-related diseases (Monteiro Junior et al., 2017). Several meta-analyses or systematic reviews indicated that exercise had a beneficial effect in reducing inflammation in patients with chronic diseases, such as breast cancer (Meneses-Echavez et al., 2016), heart disease (Hammonds et al., 2016), chronic cord injury (Neefkes-Zonneveld et al., 2015), and diabetes (Hayashino et al., 2014), but there is no consensus regarding the effect of regular practice of exercise on the circulating inflammatory biomarkers in the relatively healthy adults. In a study by Nicklas et al. the results did not find that exercise training had a significant effect on the inflammatory biomarkers (including C-reactive protein, interleukin 6, and tumor necrosis factor alpha receptor 1) in community-dwelling, older, overweight or obese sedentary adults (Nicklas et al., 2004); others also failed to present positive effects (Walker, 2010; Sahl et al., 2017). Possible reasons for this discrepancy are likely related to differences in exercise type (e.g., aerobic vs. resistance), differences between study participants (e.g., age, sex, health status, and baseline inflammation), differences in exercise protocols (e.g., intensity, frequency, and duration of intervention), or the publication of underpowered findings. Furthermore, current systematic reviews and meta-analyses have not investigated the effects of different exercise types on inflammatory markers in healthy adults, particularly middle-aged and elderly adults. A recent systematic review revealed inconsistent findings related to the effect of aerobic and resistance training on the inflammatory markers CRP and IL-6 (Cronin et al., 2017). Therefore, the present study sought to critically evaluate the effects of aerobic exercise on inflammatory makers of healthy middle-aged and elderly adults through a systematic review and meta-analysis of randomized controlled trials.

METHODS

Search Strategy

To identify eligible studies, a literature search was conducted in the electronic databases PubMed, Web of Science, the Cochrane Library, and Embase from database inception through April 4, 2018 using combinations of Medical Subject Headings (MeSH) or free text words and the concepts of aerobic exercise training, inflammatory markers, and age; the search was free of restriction to region or publication type. The reference lists of retrieved studies were also screened to identify additional relevant articles. A complete search strategy is provided in the **Supplementary Material**.

Inclusion Criteria

Studies were included in the review only if the following criteria were met: (1) Study type: Randomized controlled trials (RCTs); (2) Participants: Middle-aged and older adults (40 years and older) without a disease or medical condition; (3) Intervention: Any style of aerobic exercise or aerobic exercise combined with non-exercise interventions were performed by the experimental group for at least 4 weeks, with three or more sessions every week; (4) Control: no exercise intervention was performed expect for usual level of activity and the sham exercise (e.g., stretch, balance); (5) Outcomes: One or more inflammatory markers were measured in serum or plasma. Studies not written in English or that were without available data were excluded.

Study Selection and Data Extraction

During the preliminary screening, all searched records were imported into reference management software (NoteExpress V 3.2.0) to eliminate duplicate records and identify potential eligibility by screening titles and abstracts. Then, full-text review was performed. All discrepancies were resolved by a reviewer (GHZ). Data were extracted by one reviewer (PTQ), using a predefined form, and verified by another reviewer (GHZ). The data included the first author; study characteristics (e.g., year, design, and methodological information); participant characteristics (e.g., mean age, sample size); intervention for

the experimental and control group (e.g., duration, frequency, intensity, and style of aerobic exercise); outcome findings.

Assessment of Risk of Bias of Included Studies

The risk of bias of included studies was assessed by two independent reviewers (PTQ and RX) using the Cochrane Collaboration tool (Higgins and Green, 2011). The tool includes seven key items divided into 6 domains: (1) selective bias (random sequence generation and allocation concealment), (2) performance bias (blinding of participants and personnel), (3) detection bias (blinding of outcome assessors), (4) attrition bias (incomplete outcome data), (5) reporting bias (selective reporting), and (6) other bias. For each study, each individual item was assessed, and each domain was graded as "low," "high" or "unclear," based on whether the domain met the evaluation criteria with respect to the characteristic expressed by the items. A third reviewer (GHZ) was invited to resolve any disparities.

Data Analysis

Statistical analyses were conducted using Review Manager 5.3 software (RevMan 5.3). We conducted a meta-analysis to determine change in inflammatory markers from baseline to post-intervention by calculating the standardized mean difference (SMD) between the experimental and control groups, with a 95% confidence interval (CI). The SMD and the standard error (SE) of each inflammatory marker, both before and after treatment, were calculated utilizing Morris's formula (Morris, 2008). If the data were reported as mean and 95% CI, SD was calculated using RevMan software. If the data were reported as median interquartile range (IQR), we calculated mean and standard deviation utilizing the Wan and Luo formulae (Wan et al., 2014; Luo et al., 2015). If a study reported only the value change of the inflammatory markers, we contacted the author to obtain the original data. Data were pooled for meta-analysis when two or more studies measured the same outcome and provided data in a format suitable for pooling. The heterogeneity among the included studies was assessed using a χ^2 test and Higgins I^2 value. With the χ^2 test, $P < 0.05$ was considered to be significant. The pooled effect was calculated using the fixed-effect model when data were available, and there was no significant heterogeneity detected. Otherwise, the random-effect model was applied.

RESULTS

Study Selection

Utilizing the search strategy, 19,568 records were identified from the four electronic databases. After deleting duplicates, two reviewers (PTQ and RX) screened titles and abstracts and excluded unrelated records. Finally, 122 full-text articles were examined for eligibility, and 12 studies met the inclusion criteria (Bergström et al., 2009; Muscari et al., 2010; Tartibian et al., 2011, 2015; Friedenreich et al., 2012; Irwin and Olmstead, 2012; Nishida et al., 2015; Abdollahpour et al., 2016; Alghadir et al., 2016; Conroy et al., 2016; Sbardelotto et al., 2017; Mohammadi et al., 2018). One of these 12 studies (Sbardelotto et al., 2017) was excluded, as data remained unavailable, despite attempts to contact the original author. Thus, 11 studies were included in the review. The study selection flowchart for locating eligible articles is provided in **Figure 1**.

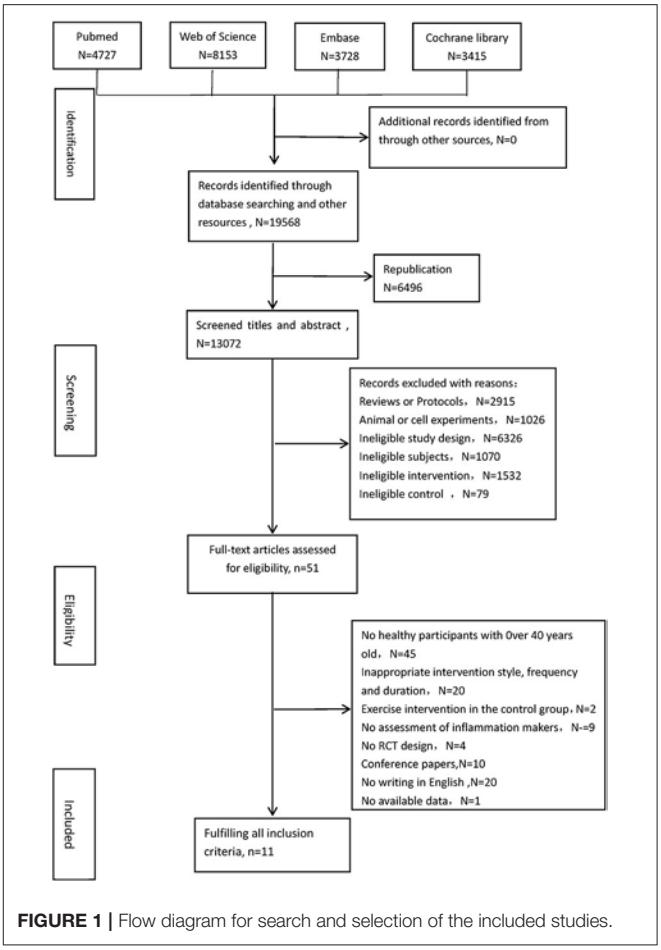

FIGURE 1 | Flow diagram for search and selection of the included studies.

Characteristics of Included Studies

The characteristics of the included articles are presented in **Table 1**. A total of 11 RCTs involving 1,250 participants (188 males and 1,062 females, age range 40–95 years) were included in the review. The styles of aerobic exercise utilized in the intervention groups were diverse, involving Tai Chi (Irwin and Olmstead, 2012), treadmill (Mohammadi et al., 2018), bench step exercises (Nishida et al., 2015), and multicomponent aerobic exercises (Bergström et al., 2009; Muscari et al., 2010; Tartibian et al., 2011, 2015; Alghadir et al., 2016); three studies (Friedenreich et al., 2012; Abdollahpour et al., 2016; Conroy et al., 2016) did not describe a specific exercise style. The duration of the exercise phase in the included studies ranged from 2 to 12 months. The frequency of exercise varied from two to five sessions weekly, with each session lasting 20–90 min. Of the 11 studies, 8 assessed exercise intensity through the heart rate reserve of 45–80% of maximum heart rate (Muscari et al., 2010; Tartibian et al., 2011, 2015; Friedenreich et al., 2012; Abdollahpour et al., 2016; Alghadir et al., 2016; Conroy et al.,

TABLE 1 | Characteristics of included studies in this systematic review.

References	Age (range)	Participant(M/F)	Intervention	Frequency, duration, and intensity of aerobic exercise	Findings
Abdollahpour et al., 2016	50–74 years	41 (0/41)	E:aerobic exercise C:maintain their usual physical activity levels	50 min/day,3 days/week for 6 months; 70–80% HRmax	↔TNF-a,↓IL-6 when E compare to C at post-test
Alghadir et al., 2016	65–95 years	100 (70/30)	E: treadmill, bicycle, and StairMaster C:maintain their usual lifestyle	45–60 min/time,3 times/week for 24 weeks; 60–70% HRmax	↔hsCRP when E compare to C at post-test
Bergström et al., 2009	45–65 years	112 (0/112)	E:brisk walk C:maintain their usual physical activity level	30 min brisk walk + 60 min aerobic exercise/day, 3 brisk walks, and 1–2 aerobic exercises/week for 24 week; at least 95% level 3–4 training intensity (Level3:1–3 walks +1 aerobic session/week; Level 4: 3 walks +2 aerobic session/week).	↔hsCRP when E compared to C at post-test
Conroy et al., 2016	50–74 years	320 (0/320)	E:aerobic exercise C: maintain their usual level of activity	45 min/session, 5 session/week, for 1 year; 70–80% HRmax	↔ IL-4, ↔ IL-10 when E compare to C at post-test
Friedenreich et al., 2012	50–74 years	210 (0/210)	E:aerobic exercise C:usual inactivity	45 min/day, 5 days/week for 1 year; 70–80% HRmax	↓hsCRP,↔TNF-α, ↔IL-6 when E compare to C at post-test
Irwin and Olmstead, 2012	59–86 years	83 (32/51)	E: Tai Chi Chih C:health education	40 min/session,3 session/week for 16 weeks; Moderate intensity	↓IL-6, ↔ hsCRP, ↔IL-18 when E compare to C at post-test
Mohammadi et al., 2018	40–60 years	24 (24/0)	E: treadmill C:usual lifestyle	20–60 min/session,3 times/week for 12 weeks; 60–70% HRmax	↔hsCRP,↔ICAM-1,↔VCAM-1 when E compare to C at post-test
Muscari et al., 2010	65–74 years	120 (62/58)	E:Cycle ergometer, treadmill and free-body activity C:health education	60 min/session, 3 times/week for 1 year; 70% HRmax	↓hsCRP when E compare to C at post-test
Nishida et al., 2015	65–85years	62 (0/62)	E:bench step exercise C:maintain their usual lifestyle	10–20 min/session,3 times/day, and a goal of 140 min/week for 12 weeks; lactate threshold	↔ IL−4,↔ IL-5,↔ IL-6, ↔ IL-8, ↔ IL-15, ↔ TNF-α, ↔ TNF-β, ↓IFN-γ when E compare to C at post-test
Tartibian et al., 2011	58–78 years	38 (0/38)	E: walking or jogging C:maintain their usual physical activity levels	25–30 min/day,3–4days/week with 45–55% HRmax for the first 12 weeks; then 40–45 min/day,4–6 days/week with 55–65% HR for the second 12 weeks	↓TNF-a, ↓IL-6, ↓PGE_2 when E compare to C at post-test
Tartibian et al., 2015	50–65 years	28 (0/28)	E:walking or jogging C:maintain their usual physical activity levels	25–30 min/day,3–4 days/week with 45–55% HRmax for the first 8 weeks; 40–45 min/day,4–6 days/week with 55–65% HR for the final 8 weeks	↓ IL-1β, ↓IL-6, ↓TNF-a, ↓hsCRP when E compare to C at post-test

E, exercise group; C, control group; ↓, significant reduction; ↔, significant increase; ↔, no change; CRP, C-reactive protein; TNF-α, tumor necrosis factor-alpha; IL-6, interleukin 6; IL-4, interleukin 4; INF-γ, interferon-gamma; PGE_2, prostaglandin E_2; IL-1β, interleukin-1beta; IL-5, interleukin-5; IL-8, interleukin-8; IL-10, interleukin-10; IL-15, interleukin-15; IL-18, interleukin-18; ICAM-1, intercellular adhesion molecule-1; VCAM-1, vascular cell adhesion molecule-1; TNF-β tumor necrosis factor-beta.

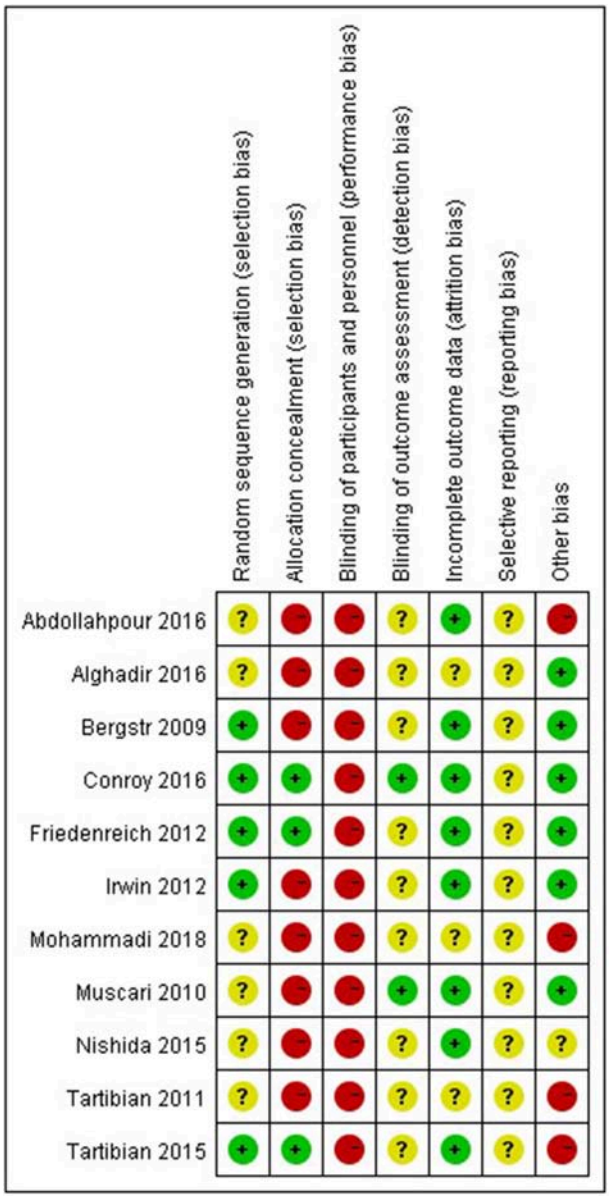

FIGURE 2 | Risk of bias in included studies: the authors' judgments on each risk of bias item for all included studies.

2016; Mohammadi et al., 2018). The remaining studies used the following strategies: (1) "at least 95% at training intensity level 3–4 (1–3 walks and 1–2 aerobic exercise/week)" (Bergström et al., 2009); (2) "moderate intensity" (Irwin and Olmstead, 2012); and (3) "an intensity of lactate threshold" (Nishida et al., 2015). All control groups were described as maintaining their usual physical activity.

Risk of Bias of the Included Studies

The risk of bias of the included studies is displayed in **Figure 2**. All of the included articles reported randomized group allocation, but only five trials (Bergström et al., 2009; Friedenreich et al., 2012; Irwin and Olmstead, 2012; Tartibian et al., 2015; Conroy et al., 2016) described the specific method of randomization, and three of those studies (Friedenreich et al., 2012; Tartibian et al., 2015; Conroy et al., 2016) reported allocation concealment. All of the included studies demonstrated high performance bias, as participants and personnel were not blind to the exercise intervention. Two studies (Muscari et al., 2010; Conroy et al., 2016) clearly described blind assessment of outcome measures. Intention-to-treat analysis was used in five studies (Bergström et al., 2009; Muscari et al., 2010; Friedenreich et al., 2012; Abdollahpour et al., 2016; Conroy et al., 2016), and three studies (Irwin and Olmstead, 2012; Nishida et al., 2015; Tartibian et al., 2015) described reasons why participants dropped out or failed to follow up. The risk of other bias in four studies was judged as "high" due to limited sample size (Tartibian et al., 2011, 2015), disproportionate dropout rates between the groups (Abdollahpour et al., 2016), and lack of baseline measurements (Mohammadi et al., 2018).

Effect of Interventions

Pre-intervention and post-intervention data were reported using mean with standard deviation (SD), or mean with 95% confidence interval, in 10 studies (Muscari et al., 2010; Tartibian et al., 2011, 2015; Friedenreich et al., 2012; Irwin and Olmstead, 2012; Nishida et al., 2015; Abdollahpour et al., 2016; Alghadir et al., 2016; Conroy et al., 2016; Mohammadi et al., 2018), and median (IQR) or median (range) in one study (Bergström et al., 2009). Of the 15 inflammatory markers measured in the included studies, only CRP, TNF-α, IL-6, and IL-4 were reported in more than two trials, rendering the data suitable for meta-analysis.

The effect of aerobic exercise on levels of CRP was measured in seven studies. Seven studies (Bergström et al., 2009; Muscari et al., 2010; Friedenreich et al., 2012; Irwin and Olmstead, 2012; Tartibian et al., 2015; Alghadir et al., 2016; Mohammadi et al., 2018) with 774 participants reported appropriate data. Three studies (Muscari et al., 2010; Friedenreich et al., 2012; Tartibian et al., 2015) demonstrated a significantly lower post-intervention level of CRP in the exercise group compared to the control group. The pooled SMD showed a statistically significant decrease in CRP (SMD = 0.53, 95% CI 0.26–0.81, $P = 0.0002$; **Figure 3**).

Five of the included studies (Tartibian et al., 2011, 2015; Friedenreich et al., 2012; Nishida et al., 2015; Abdollahpour et al., 2016) looked at TNF-α levels, and two of these five(Tartibian et al., 2011, 2015) found a significant post-intervention reduction of the level of TNF-α in the exercise group compared with the control group; however, when the data from these five studies were pooled, SMD between the studies demonstrated a significant reduction (SMD = 0.75, 95% CI 0.31–1.19, $P = 0.0007$; **Figure 4**).

Six of the included studies investigated IL-6 (Tartibian et al., 2011, 2015; Friedenreich et al., 2012; Irwin and Olmstead, 2012; Nishida et al., 2015; Abdollahpour et al., 2016), and four (Tartibian et al., 2011, 2015; Irwin and Olmstead, 2012; Abdollahpour et al., 2016) of these studies identified a significant reduction in IL-6 levels for the aerobic exercise group compared to the control group. The forest plots also showed aerobic exercise significantly reduced IL-6 levels in healthy middle-aged and older adults (SMD = 0.71, 95% CI 0.24–1.17, $P = 0.003$; **Figure 5**).

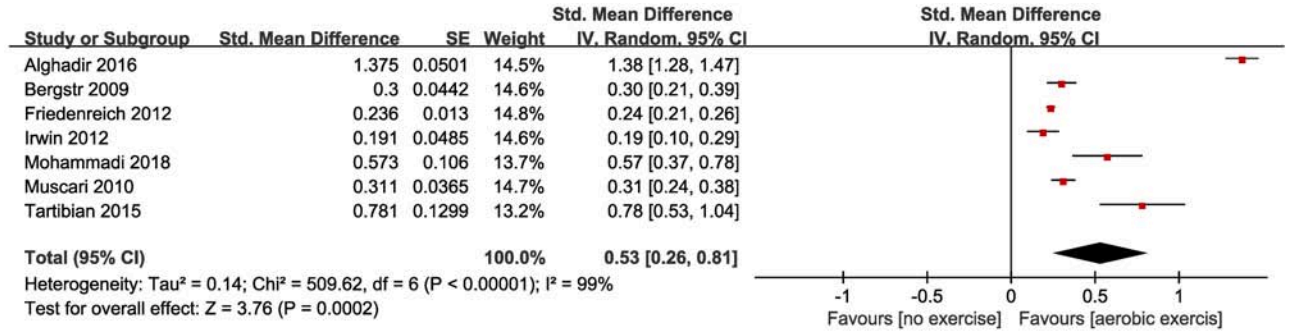

FIGURE 3 | Forest plots of the effect of aerobic exercise on CRP compared with no exercise.

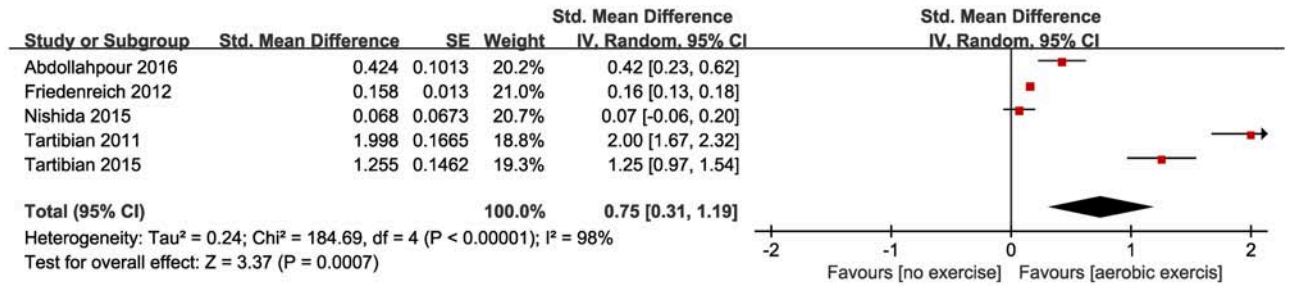

FIGURE 4 | Forest plots of the effect of aerobic exercise on TNF-α compared with no exercise.

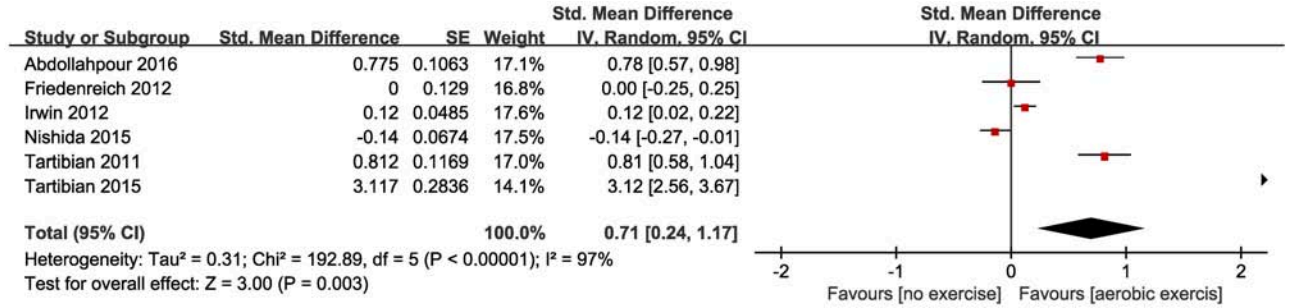

FIGURE 5 | Forest plots of the effect of aerobic exercise on IL-6 compared with no exercise.

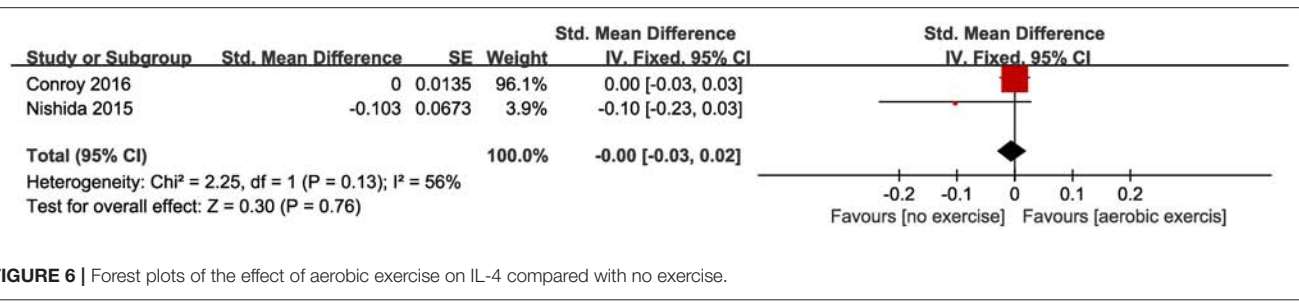

FIGURE 6 | Forest plots of the effect of aerobic exercise on IL-4 compared with no exercise.

Only two of the eligible studies (Nishida et al., 2015; Conroy et al., 2016) reported IL-4 levels, and neither reported a statistically significant decrease in IL-4 levels for the aerobic exercise group compared with the control group. Likewise, the pooled SMD did not show significant changes between the aerobic exercise and control groups (SMD = 0.00, 95% CI −0.03 to 0.02, $P = 0.76$; **Figure 6**).

Three studies (Tartibian et al., 2011, 2015; Nishida et al., 2015) that measured the concentration of interferon-gamma (INF-γ), prostaglandin E_2 (PGE_2), and interleukin-1beta (IL-1β), respectively, demonstrated a statistically significant reduction in the levels of these markers for the aerobic exercise group compared to controls; other studies (Irwin and Olmstead, 2012; Nishida et al., 2015; Conroy et al., 2016; Mohammadi et al., 2018), involved individually in assessing interleukin-5 (IL-5), interleukin-8 (IL-8), interleukin-10 (IL-10, interleukin-15 (IL-15), interleukin-18 (IL-18), intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and tumor necrosis factor-beta (TNF-β) levels, failed to find statistically significant differences between the aerobic exercise groups and controls.

Adverse Events

No adverse events were reported in the studies included in this review.

DISCUSSION

This systematic review and meta-analysis sought to evaluate the relationship between aerobic exercise and inflammatory markers in healthy middle-aged and older adults. Eleven randomized controlled trials, with a total of 1,138 participants, that compared aerobic exercise groups with no-exercise control groups were included in this review. The results demonstrate that regular aerobic exercise has a positive effect on decreasing most of the reported inflammatory makers, including CRP, TNF-α, and IL-6, in healthy middle-aged and older adults.

There are strong correlations between inflammation and aging. The prospective epidemiological studies have found an increased risk of age-related chronic diseases is associated with increased basal-level inflammation (Ridker et al., 2000). As a result, aging is associated with low-grade inflammation (Nagano et al., 2005), and increases in circulating levels of inflammatory markers have been observed in the aged populations (Ogawa et al., 2008). It is established that with age, physiological processes generate adaptations within the immune system, resulting in a continuous response of factors that trigger a chronic inflammatory response (Ostan et al., 2008), including increased fat tissue, decreased sex steroid production, and chronic disorders (Woods et al., 2012). Exercise has direct effects on the cellular immune system, as cytotoxic immune cells can be mobilized into circulation through adrenergic signaling during exercise performance (Idorn and Hojman, 2016). Therefore, exercise is recommended as an appropriate non-pharmacological strategy to modulate the systemic inflammatory status; however, many previous reviews have been limited to studying exercise effects on inflammation in individuals with disease (Hayashino et al., 2014; Neefkes-Zonneveld et al., 2015; Hammonds et al., 2016; Meneses-Echavez et al., 2016). A recent systematic review reported regular exercise training may decrease levels of CRP and IL-6, but no statistical significance was found for TNF-α in an elderly population (Monteiro Junior et al., 2017). Another meta-analysis, not limited to a specific clinical population, indicated that exercise training could lower CRP levels in individuals (Fedewa et al., 2017). However, considering the methodological diversity among the included studies, particularly the differences between exercise types (i.e., aerobic, resistance) and different basal levels of cytokines, the results of those meta-analysis or review should be interpreted cautiously. The present systematic review focused on the effect of aerobic exercise on inflammatory markers in healthy middle-aged and older adults. Following the initial screening of over 13,000 article references, 11 randomized controlled trials were ultimately included in the review. The pooled results revealed aerobic exercise led to a significant reduction of inflammatory markers, including CRP, TNF-α, and IL-6. Furthermore, all included studies adhered to a pretest-posttest-control design, in which participants are randomly assigned to the treatment or control group, and each participant is measured both before and after the treatment. Considering that preexisting differences between groups can artificially inflate or obscure posttest differences, we used the Morris method to adjust the heterogeneity of inflammatory markers at baseline (before treatment) among the included studies (Morris, 2008). Therefore, the results in this review were more robust than findings of previous studies.

This study has several limitations. First, significant heterogeneity was found in the CRP, TNF-α, and IL-6 data among the included studies; a possible cause may be that inflammatory marker levels are affected by different methods of collection and sample preparation, as well as by time elapsed between previous exercise session and plasma or serums measurement (Wu et al., 2007). Additionally, there were discrepancies between the included studies regarding different types, duration, and frequencies of the aerobic exercise interventions. These are all potential sources of differences in results between studies. However, due to an insufficient number of studies of each inflammatory marker, sub-analysis could not be performed. Second, the pooled effect for other inflammatory markers, such as IL-8, IL-10, and VCAM-1, could not be analyzed due to an insufficient number of included studies that assessed these markers. Thus, additional randomized controlled trials should be conducted to determine the effects of aerobic exercises on other inflammatory markers. Third, studies not reported in English were excluded, which could have led to publication bias; however, funnel plot analysis to detect publication bias was not possible due to the limited number of included studies (less ten studies for each outcomes). Finally, relating to the specificity of intervention, it is not feasible to blind participants and exercise trainers during aerobic exercise intervention; therefore, performance bias may be inevitable.

CONCLUSIONS

This review reveals that aerobic exercise has significant benefits on levels of CRP, TNF-α, and IL-6. Considering the limited number of included studies, considerably larger-sample size

RCTs are necessary to determine the effect of aerobic exercise on additional inflammatory markers in middle-aged and older adults.

AUTHOR CONTRIBUTIONS

LC, GZ, and JT conceived and designed the study. PQ, RX, HL, and BY performed the search, extraction of data, and methodological assessment. PQ and GZ analyzed the data and wrote the paper. All authors read and approved the final manuscript.

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SUPPLEMENTARY MATERIAL

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Long-Term Endurance and Power Training May Facilitate Motor Unit Size Expansion to Compensate for Declining Motor Unit Numbers in Older Age

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The evidence concerning the effects of exercise in older age on motor unit (MU) numbers, muscle fiber denervation and reinnervation cycles is inconclusive and it remains unknown whether any effects are dependent on the type of exercise undertaken or are localized to highly used muscles. MU characteristics of the vastus lateralis (VL) were assessed using surface and intramuscular electromyography in eighty-five participants, divided into sub groups based on age (young, old) and athletic discipline (control, endurance, power). In a separate study of the biceps brachii (BB), the same characteristics were compared in the favored and non-favored arms in eleven masters tennis players. Muscle size was assessed using MRI and ultrasound. In the VL, the CSA was greater in young compared to old, and power athletes had the largest CSA within their age groups. Motor unit potential (MUP) size was larger in all old compared to young ($p < 0.001$), with interaction contrasts showing this age-related difference was greater for endurance and power athletes than controls, and MUP size was greater in old athletes compared to old controls. In the BB, thickness did not differ between favored and non-favored arms ($p = 0.575$), but MUP size was larger in the favored arm ($p < 0.001$). Long-term athletic training does not prevent age-related loss of muscle size in the VL or BB, regardless of athletic discipline, but may facilitate more successful axonal sprouting and reinnervation of denervated fibers. These effects may be localized to muscles most involved in the exercise.

Keywords: exercise, motor unit, muscle, master athlete, electromyogarphy

INTRODUCTION

Voluntary movements are controlled through precise activation of motor unit (MU) populations. A single MU is composed of an alpha motor neuron with its cell body in the ventral horn of the spinal cord, an axon, and all of the muscle fibers innervated by that single motor neuron. Conditions leading to declining MU numbers and alterations of MU sizes, such as advancing older age, may impact upon many aspects of neuromuscular function and physical performance. Estimates made from cadaveric specimens showed around 30% fewer motor neuron cell bodies in the lumbo-sacral spinal cord of older compared with younger adults (Kawamura et al., 1977; Tomlinson and Irving, 1977; Mittal and Logmani, 1987). Electrophysiological techniques using surface and intramuscular electromyography (EMG) also report 30–50% fewer MUs by the age of 70 years in human limb muscles (McNeil et al., 2005; Piasecki et al., 2016a,c). Whilst the age-related reductions in neuromuscular control are multifactorial including alterations in MU firing rate (Dideriksen et al., 2012), proprioception and afferent feedback (Baudry, 2016), the lower MU numbers observed in older age directly coincides with loss of muscle fibers (Lexell et al., 1988; McPhee et al., 2018) and contributes to sarcopenia defined by low muscle mass and functional impairments.

We have shown that the loss of MUs occurs relatively early into older age and precedes sarcopenia (Piasecki et al., 2016c) because not all of the muscle fibers of affected MUs are lost; some may be "rescued" by axonal sprouting of adjacent surviving neurons, thereby helping to preserve total muscle mass. As a consequence, older muscle has fewer MUs but they are larger in size (Hepple and Rice, 2016; Piasecki et al., 2016b). The relative success of sprouting may differ between people and influence the rate of muscle declines during aging. For example, the reinnervation process may be less successful in sarcopenic individuals with the smallest muscle mass in older age (Piasecki et al., 2018c). Thus, finding an intervention to minimize motor neuron loss and associated muscle fiber denervation, and/or increasing axonal sprouting to rescue denervated fibers would preserve muscle mass and function for longer into older age. Regular intense exercise is one possible intervention.

There is conflicting evidence concerning the potential benefits of regular intense exercise to preserve MU numbers and enhance axonal sprouting. One cross-sectional study showed higher MU numbers in the tibialis anterior (TA) of athletic old compared with non-athletic age-matched controls (Power et al., 2010), but MU numbers of the biceps brachii (BB) were not maintained (Power et al., 2012), suggesting these effects are not systemic, but are localized to the highly used muscles. However, a similar cross-sectional study showed no such preservation of MU numbers in the TA of older runners (Piasecki et al., 2016a). Previous studies in this field have lacked useful comparison between groups by failing to include highly athletic young as comparators for the athletic old and not distinguishing power or endurance athletes (Power et al., 2010; Piasecki et al., 2016a). This is a problem because it is not known whether high performance younger athletes have different MU numbers or sizes to non-athletic young, or if one discipline may differ from another. Therefore, when investigating associations between aging, exercise and MU characteristics, it is more appropriate to include athletic old and athletic young adults competing in endurance and power disciplines, along with age-matched controls. An alternative approach is to compare muscles within the same person after long-term discordance of exercise exposure between limbs, as occurs with racquet sports such as tennis.

The aims of the present study were to determine whether estimated MU numbers and motor unit potential (MUP) sizes differ between young power and endurance athletes, and whether decades of exercise into older age preserves MU numbers and increases MUP size. Three main hypotheses were tested. Firstly, that masters athletes would have *fewer* vastus lateralis (VL) MUs than young athletes and similar to age-matched non-athletes. This would indicate that exercise training does not prevent typical age-related MU loss. Secondly, that masters athletes would have *larger* MUPs than young athletes and age-matched non-athletes. This would indicate that exercise enhances reinnervation of denervated fibers to increase MU size. Finally, to account for any possibility of secular changes affecting MU numbers or sizes in cross-sectional studies and to examine the notion of localized influences on MU plasticity, we hypothesized that the favored racquet arm of masters tennis players would have similar MU numbers, but larger MUP sizes compared with the non-favored arm.

MATERIALS AND METHODS

Participants and Ethical Approval

The study was conducted in accordance with the *Declaration of Helsinki* and approved by the Manchester Metropolitan University Research Ethics Committee and the National Research Ethics Service Committee Northwest (15/NW/0426). All participants provided written informed consent. Eighty-five young and older males participated in the first part of this study. This included 15 young controls, 8 young endurance athletes, 11 young power athletes, 22 older controls, 18 master endurance athletes and 11 master power athletes. Eleven older male tennis players took part in the second part of the study. The young and old controls were recruited from the University population and the local community; they were recreationally active but did not compete in any sports. All athletes (young and old) were recruited from running clubs, two national masters athletics competitions and from an advertisement placed in a national athletics magazine.

For part two of the study, masters tennis players were recruited from the British Seniors' Indoor Tennis Championships and tested onsite at the competition location.

Athlete Status

Part 1: All of the athletes were actively competing in their respective sports and distances at the time of testing and all completed more than 5 h of training per week specific to their sport or discipline. All of the master athletes had trained specifically for their events since young adulthood (>18 years) and the median number of years of training at the point of

testing was 46 years for the endurance athletes and 51 years for the power athletes. All young athletes had trained specifically for their respective events for a minimum of 5 years prior to testing. The age-graded performance (AGP) of an athlete allows a comparison of the athlete against the current world record within their age group and discipline. It is expressed as a percentage of the world record. All of the young endurance athletes competed at distances of 5000 m and above and had a mean AGP of 87.2 (2.1). The young power athletes consisted of a combination of competitive Olympic weightlifters and power lifters and had a mean AGP of 67.9 (3.4). The old endurance athletes all competed at distances of 3000 m and above; this group had a mean AGP of 79.1 (9.2) and included four athletes ranked in the top three in Great Britain for their respective ages and distances. The old power athletes all competed in sprinting distances of 400 m and below, and the mean AGP was 84.5 (9.9). This group included two athletes ranked in the top three in Great Britain for their respective ages and distances.

Part 2: Age-graded performances are not available for the masters tennis players. All athletes had trained and competed in tennis prior to the age of 50 years and were recruited and tested at a senior national competition.

Anthropometric Assessments

Part 1: The cross-sectional area (CSA) of the VL at the motor point (approximately mid muscle belly) was measured in the right leg with magnetic resonance imaging (MRI) using a T1-weighted turbo 3D sequence on a 0.25-T G-Scan with the participants lying supine (Esaote, Genoa, Italy). The motor point was marked using an external reference taped to the skin that was a high-fat capsule and easily identifiable from an MRI scan. Contiguous transverse-plane slices of 6 mm thickness were collected. Images were exported and analyzed off-line as previously described, using Osirix imaging software (Osirix medical imaging, Osirix, Atlanta, GA, United States (Maden-Wilkinson et al., 2014; **Figure 1**). Body mass and height were measured using calibrated scales and stadiometry, respectively, and body mass index (BMI) calculated. Total body fat percentage was assessed by dual-energy X-ray absorptiometry (Lunar Prodigy Advance, version EnCore 10.50.086; GE Healthcare, Little Chalfont, United Kingdom) with the participant lying supine with legs and arms fully extended.

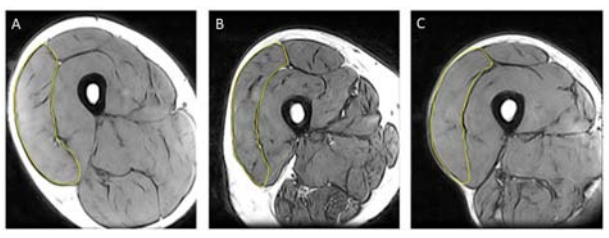

FIGURE 1 | Representative MRI images highlighting vastus lateralis (VL) cross sectional area (CSA) from **(A)** young control; **(B)** older control; and **(C)** old endurance athlete.

Part 2: The BB thickness was measured in both favored and non-favored arms using B-mode ultrasound (Esaote MyLab25, Genoa, Italy) with a 7.5 MHz linear probe held perpendicular to the BB muscle belly. The arm and forearm were relaxed and fully extended at 180°, supported at the shoulder and elbows, with the arm in the supinated position and palm of the hand facing anteriorly. The greatest distance from the superficial to the deep aponeurosis of BB was taken as the muscle thickness.

Strength Assessments

Part 1: Right knee extensor strength was assessed with participants sitting with hips and knees flexed at 90° and the leg securely fastened to a force transducer 30 cm below the center of the knee joint. To familiarize with the equipment and to "warm-up" the muscle, participants performed a series of submaximal contractions. They were then instructed to perform a maximal isometric contraction, accompanied by verbal encouragement and visual feedback of force on a computer screen. This was repeated three times, with 60 s rest intervals. The best effort was taken as maximum voluntary isometric contraction force (MVC).

Part 2: In the masters tennis players, participants were seated with the shoulder abducted in the lateral plain to 90°. The height of the chair was adjusted so the participant could sit comfortably with the triceps resting on the base of the custom-built dynamometer. The elbow was flexed at 110° and the forearm was attached to the dynamometer around 27 cm from the tip of the elbow, with supination of the palm. Participants were familiarized to elbow flexion by performing three isometric contractions lasting 4 s at around 80% of maximal effort. Next, isometric elbow flexion MVC was assessed three times with 60 s rest between efforts. The highest value was accepted as MVC.

Identifying the Motor Points

The motor point of the muscle is defined as the area of muscle providing the largest twitch from the smallest percutaneous stimulus (Botter et al., 2011). Motor points were identified using a cathode probe (Medserve, Daventry, United Kingdom) to apply percutaneous electrical stimulation at 400 V, pulse width of 50 μs, and current of around 8 mA, (DS7A Digitimer, Welwyn Garden City, Hertfordshire, United Kingdom). A self-adhesive electrode (Dermatrode, Farmadomo, NL) was used as the anode.

Part 1: The proximal motor point of the VL was located around 22 cm proximal to the lateral femoral condyle, close to the midline of the VL. The anode was placed over the right gluteus.

Part 2: The motor point of the BB was located around 14 cm from the medial epicondyle, and around 20 mm medially from the centreline of the two heads of the BB. The anode was placed over the acromion process of the scapula on the same side as the tested BB.

Surface EMG

Part 1: The active recording sEMG electrode (disposable self-adhering Ag-AgCl electrodes; 95 mm^2, Ambu Neuroline, Baltorpbakken, Ballerup, Denmark) was placed over the motor point and positioned to give the largest compound muscle action potential (CMAP, sometimes known as the M-wave) and shortest rise-time in response to stimulating the motor nerve. A reference

electrode was placed over the patella tendon and a common ground electrode was placed over the patella for VL.

Part 2: For the BB, the active recording electrode was placed over the motor point, a reference electrode was placed over the lateral epicondyle and a common ground electrode was placed over the elbow.

In all cases the common ground electrode served for both surface and intramuscular EMG (iEMG) measurements. Surface EMG signals were bandpass filtered between 5 Hz and 5 kHz via CED 1902 amplifiers (Cambridge Electronics Design Ltd., Cambridge, United Kingdom). Signals were digitized with a CED Micro 1401 data acquisition unit (Cambridge Electronic Design). The sEMG signals were sampled at 5 kHz.

Compound Muscle Action Potential

Part 1: Compound muscle action potentials were evoked using a manually triggered stimulator (model DS7AH; Digitimer). For the VL the anode was over the right gluteus, and the cathode probe over the femoral nerve, approximately half way between the anterior superior iliac spine and the pubic tubercle, proximal to the groin crease, but distal to the inguinal nerve.

Part 2: A bar electrode with the anode and cathode spaced 3 cm apart (Model MLADDF30; AD Instruments, Oxford, United Kingdom) was held over the musculotaneous nerve.

For both muscles the current was increased incrementally until the CMAP amplitude plateaued, generally between 100–200 mA. The current was then increased by 30 mA to ensure supra-maximal stimulation.

Intramuscular EMG

After determining the MVC and CMAP, a concentric needle electrode (Model N53153; Teca, Hawthorne, NY) was inserted, immediately adjacent to the recording surface electrode over the motor point, to a depth of 1.5 – 2 cm into the VL or 1 – 1.5 cm into the BB. The iEMG signals were bandpass filtered from 10 to 10 kHz and sampled at 25 kHz. The force and EMG signals were displayed in real-time using Spike2 software (v8.01) and data were stored for off-line analysis.

Recording From Individual Motor Units During Voluntary Contractions

The participant performed a voluntary, low force contraction while the needle position was adjusted to obtain intramuscular MUPs with peak second derivative values >5 kV/s^2, thus ensuring the recording needle electrode was proximal to fibers belonging to the sampled MUs (Stashuk, 1999b). The participant then performed a voluntary contraction lasting 12–15 s, keeping as close as possible to a target line shown on the computer monitor that was set at 25% MVC with real-time visual feedback. The needle electrode was then repositioned by combinations of rotating the bevel 180° and withdrawing it by 2–5 mm. The procedure of needle positioning, voluntary contraction and signal recording was repeated until a minimum of six recordings from varying depths had been obtained to sample from representative sets of MUs. The participant rested for 30 s between contractions.

EMG Analysis and Motor Unit Number Estimates

The procedures for recording and analyzing individual MUPs and calculating motor unit number estimates (MUNE) values have been described in detail previously (Piasecki et al., 2016a,c). Briefly, intramuscular and surface EMG signals were analyzed using decomposition based quantitative electromyography (DQEMG) (Boe et al., 2005, 2006; Stashuk, 1999a) (see **Figures 2**, **3** for iEMG raw data). MUNE values were computed as the ratio of the size of the CMAP to the size of a mean surface MUP (sMUP). Surface MUPs provide a surface EMG-based representation of a sampled MU. A sMUP is calculated using ensemble-averaging of suitable segments of the surface EMG signal, identified using the MUPs from motor unit potential trains (MUPTs) of the separate MUs (**Figure 3**). MUPTs that had fewer than 40 MUPs were excluded. The mean number of MUPs contributing to make a corresponding sMUP here was 111 ($\pm$ 25, range 60 – 336). The mean sMUP was obtained by ensemble-averaging all of the negative-peak-onset-aligned sMUPs of the MUs sampled in a muscle. For the VL, CMAP and sMUP size were quantified by the negative peak area. For the BB, 2 of the participants had excessive CMAP durations which artificially inflated CMAP area, therefore CMAP and sMUP size were represented using negative peak amplitude (**Figure 4**). Both area and amplitude values have been used in previous studies to represent CMAP and sMUP size and areas and amplitudes sampled from the same muscle are strongly correlated.

A MUNE value was calculated by dividing the CMAP area or amplitude by the area or amplitude of an ensemble-averaged mean sMUP. This method was developed for the study of small peripheral muscles where the entire muscle may be within the capture area of the surface electrode. However, this does not account for differences in muscle size therefore the intramuscular MUNE (iMUNE), calculated as MUP size normalized to muscle CSA, is also reported (Piasecki et al., 2018b). Values obtained by both methods are obtained from a single contraction intensity (25% MVC), and may be considered as an index which is positively correlated with the number of MUs within the muscle, but is not as an actual number of MUs (Piasecki et al., 2018b).

Statistical Analysis

Differences between groups in part one of the study were evaluated using a two-way ANOVA (2 $\times$ 3 factorial design) with *age* and *discipline* as fixed factors. Where significant interactions between the effects of age and discipline were observed, interaction contrasts were performed to reveal the cause of the interaction. The contrast estimate represents the "difference between the differences"; in this case it shows how the age-related contrasts differ across the three disciplines. For example, comparing the difference between young and old *controls* with the difference between young and old *endurance* athletes is calculated as: difference between young and old controls *minus* the difference between young and old endurance athletes.

The interaction contrasts are displayed in brackets as a contrast estimate, 95% CI, *p*-value. Also, where a

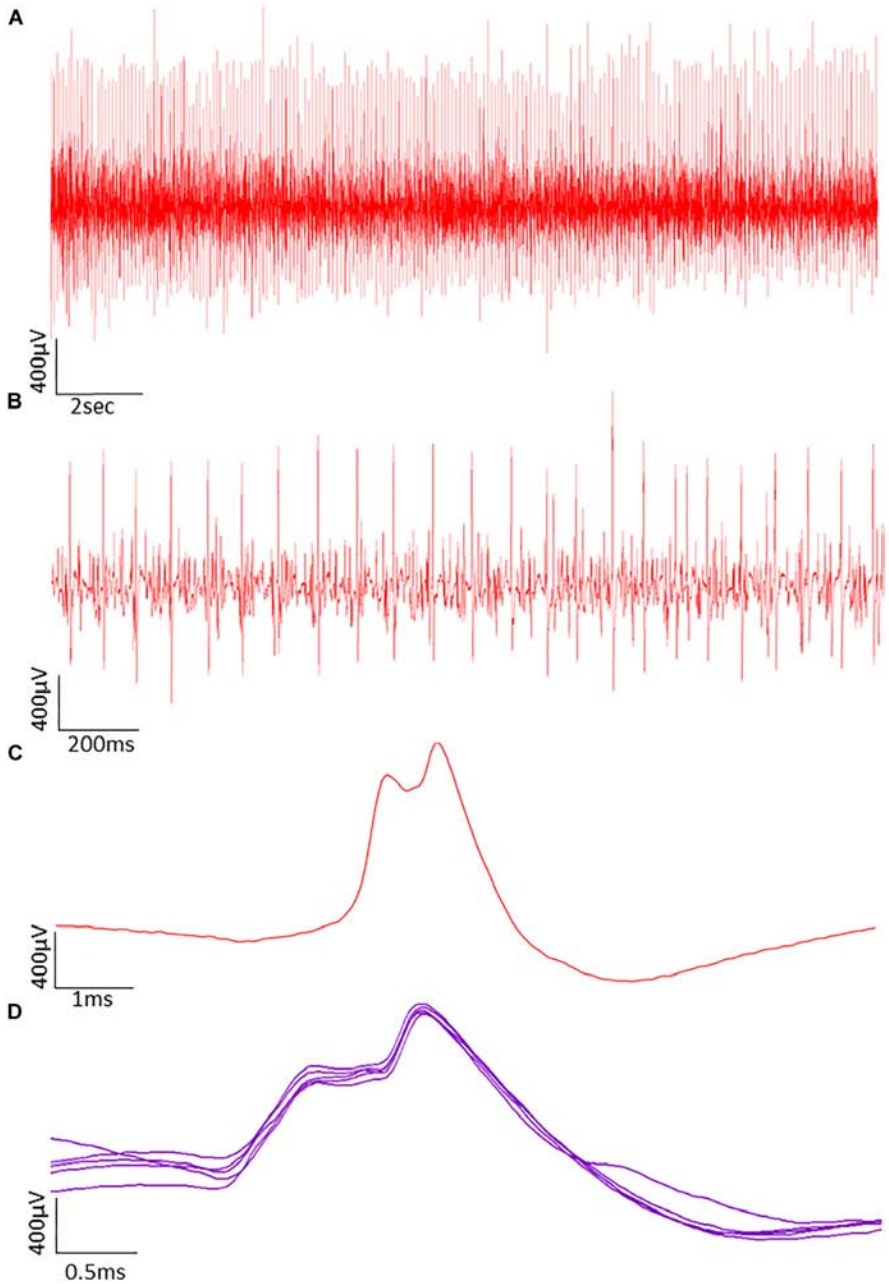

FIGURE 2 | Typical raw data showing motor unit potentials (MUPs) recorded by intramuscular EMG. The MUPs were recorded from an older power athlete holding a sustained isometric contraction at 25% MVC. **(A)** MUPs from populations of active motor units. **(B)** Data from A shown with a reduced time window to reveal more detail. **(C)** A Single MUP isolated from the traces shown in B. **(D)** Traces of the same MUP overlaid from consecutive firing.

significant interaction was observed, *simple* main effects of *age* and simple main effects of *discipline* with pairwise comparisons and Bonferroni adjustments are reported. Where no significant interactions between the effects of *age* and *discipline* were observed, the main effects of *age* and *discipline* are reported. In part two of the study opposite arms was compared using a paired samples *t*-test. Data were log transformed when not normally distributed. Analysis was performed using SPSS Version 21 (SPSS, Chicago, IL) software and $p \leq 0.05$ was considered statistically significant.

RESULTS

Athlete Participant Characteristics

Eighty-five participants were recruited into six sub-groups according to age and exercise discipline. Participant

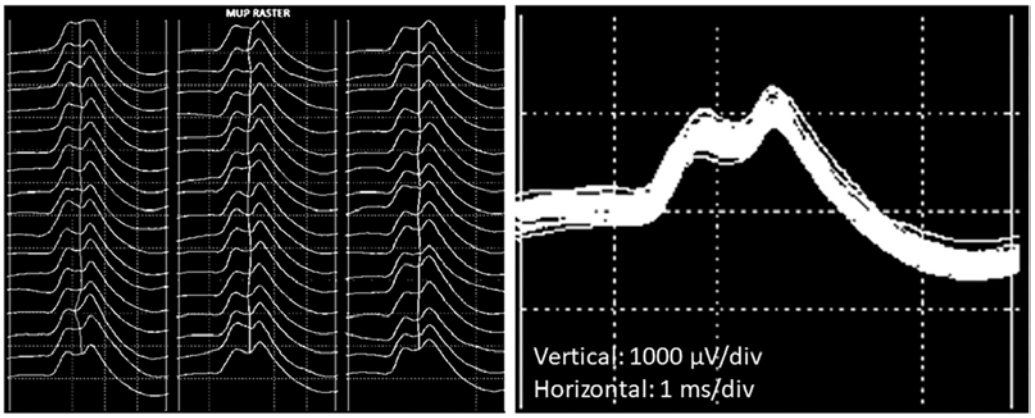

FIGURE 3 | Example images of a single motor unit potential decomposed from intramuscular EMG recordings from the VL. (Left) Raster plot of a motor unit potential train (MUPT) showing 51 consecutive firings from the same MU. (Right) Shimmer plot with the MUPs from the left-hand image overlaid.

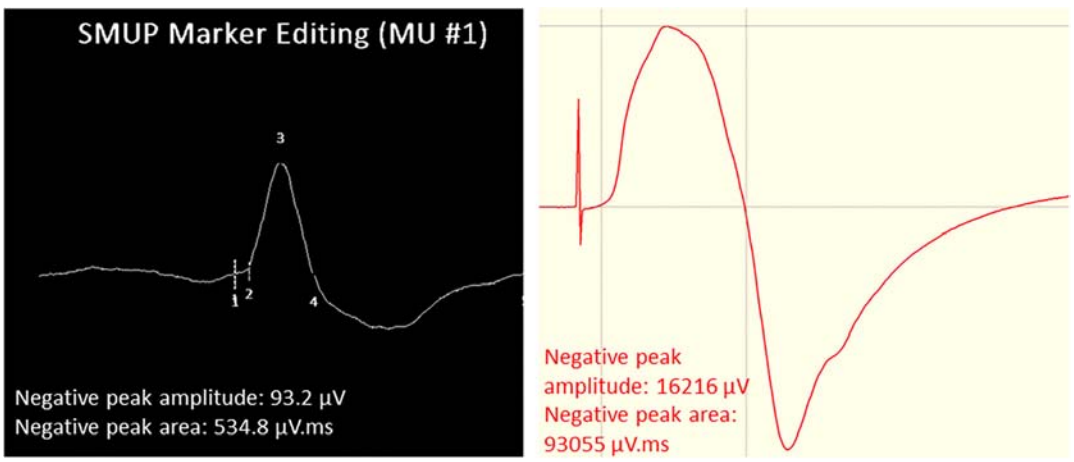

FIGURE 4 | Example images of VL surface EMG measurements. Left: Surface motor unit potential (sMUP) averaged from 124 observations of the same MUP. Numbers indicate; 1, negative peak onset; 2, onset of intramuscular MUP; 3, negative peak amplitude; 4, negative peak end. (Right) Compound muscle action potential (CMAP) obtained from percutaneous electrical stimulation of the femoral nerve. Initial spike reflects the stimulus artifact.

TABLE 1 | Participant characteristics by age and exercise discipline.

	Young control (n = 15)	Young endurance (n = 8)	Young power (n = 11)	Old control (n = 22)	Old endurance (n = 18)	Old power (n = 11)	P-value for interaction	P-value for main effects between study groups	
							Age x Discipline	Age difference	Discipline difference
Age (yrs)	25.8 (4.9)	25.1 (4.3)	28.2 (3.2)	70.3 (3.6)	69.4 (3.9)	70.5 (6.7)	0.626	**<0.001**	0.1281
Height (m)	1.78 (0.07)	1.78 (0.04)	1.80 (0.05)	1.75 (0.06)	1.73 (0.06)	1.74 (0.07)	0.682	**0.002**	0.757
Body mass (kg)	74.7 (9.4)	65.7 (5.6)[a]	91.5(8.6)[a,b]	73.9 (6.8)	65.5 (6.9)[a]	74.6 (8.0)[b,c]	**<0.001**	**–**	**–**
BMI (kg/m^2)	23.3 (2.7)	20.7 (1.3)[a]	28.2 (2.9)[a,b]	24.3 (1.9)	21.4 (1.6)[a]	24.7(2.5)[b,c]	**0.001**	–	**–**
Body Fat (%)	18.9 (7.5)	9.3 (3.9)	16.5 (7.9)	22.4 (5.3)	13.9 (5.5)	18.1 (5.9)	0.813	**0.037**	**<0.001**
Knee extensor MVC (N)	591 (148)	459 (159)	681 (110)	350 (99)	314 (83)	485 (52)	0.183	**<0.001**	**<0.001**

Main effects of age and discipline are shown in final columns where there was no significant interaction between these effects. Where this interaction was significant, simple main effects of discipline are shown as: [a]indicates difference to control within age group. [b]indicates difference to endurance within age group. Simple main effects of age are shown as [c]indicates young vs. old within-discipline difference. Abbreviations: m, meters; kg, kilograms, BMI, body mass index, ALM, appendicular lean mass, CSA, cross sectional area; MVC, maximal voluntary contraction. P-values for significant differences are shown in bold.

characteristics are shown in **Table 1**. The only statistically significant interactions between the effects of age and discipline were for body mass ($p < 0.001$) and BMI ($p = 0.001$) due to the age-related contrasts (comparing young and old of the same discipline) being greater for power athletes compared to endurance or controls (both $p < 0.001$).

Older men had a higher body fat % than younger men. Body fat % also differed significantly across disciplines, with controls having highest fat %, followed by power athletes and then endurance athletes with lowest. Older men had a lower knee extensor MVC than younger men. Knee extensor MVC also differed significantly across disciplines, as it was highest in power athletes, followed by controls, then endurance athletes (**Table 1**).

Muscle Size

There was a significant interaction between the effects of age and discipline in VL CSA (**Figure 5**, $p = 0.001$ for interaction).

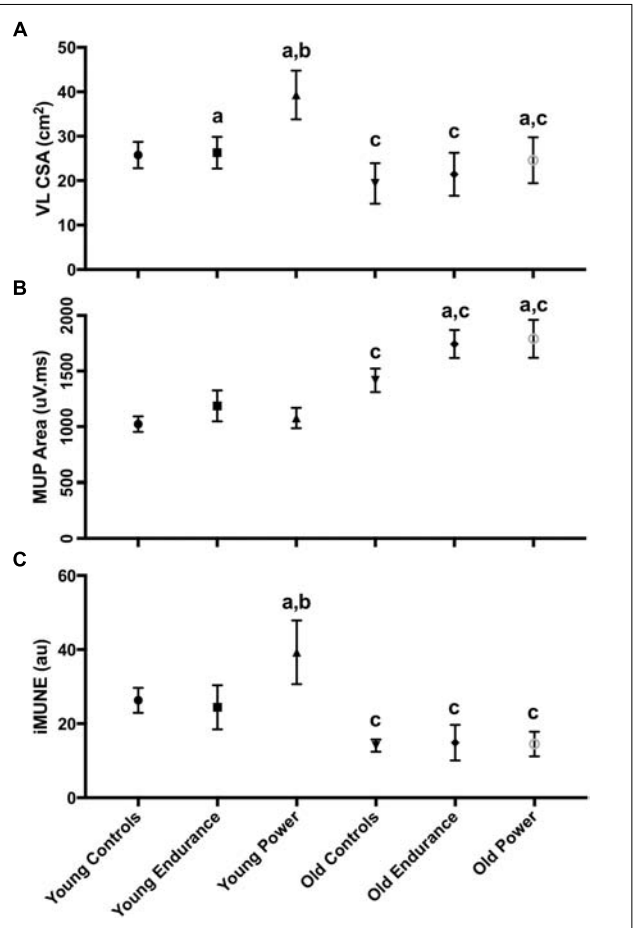

FIGURE 5 | Neuromuscular characteristics of the VL. Error bars indicate 95% CI. VL iMUNE calculated as mean MUP normalized to VL CSA. Simple main effects: *a* indicates significant difference to control, within age group. *b* indicates significant difference to endurance, within age group. *c* indicates significant young vs. old within-discipline difference. Abbreviations: VL, vastus lateralis; CSA, cross sectional area; MUP, motor unit potential; iMUNE, intramuscular motor unit number estimate.

Interaction contrasts revealed the age-related differences in VL CSA were greater in the power athletes than controls: the difference between young and old controls minus the difference between young and old power athletes was 8.28 cm^2 (95% CI 3.29 to 13.26, $p = 0.001$); the difference between young and old controls minus the difference between young and old endurance athletes was 9.81 cm^2 (95% CI 4.36 to 15.25, $p = 0.001$). There was no significant difference between values for young and old controls compared with young and old endurance athletes (1.53 cm^2, 95% CI −3.23 to 6.29, $p = 0.525$). The VL CSA was larger in young than old in all three groups (all $p < 0.001$). VL CSA differed significantly across disciplines ($p < 0.001$) and was greater in the young compared with the older participants ($p = 0.011$). Pairwise comparisons showed the young controls did not differ from young endurance athletes ($p > 0.99$) but the young endurance and young controls had smaller VL CSA than young power (both $p < 0.001$). The old controls did not differ from old endurance ($p = 0.494$) but had significantly smaller VL CSA than old power ($p = 0.009$), while old endurance and old power did not differ significantly ($p = 0.241$) (**Figure 5**).

Athlete CMAP, sMUP, and Estimates of MU Number

There were no statistically significant interactions between the effects of age and discipline on CMAP or sMUP area. The younger men had 22–50% larger CMAP area than old, with no difference across disciplines. The sMUP area did not differ by age or by discipline. As with CMAP, younger men had 18–38% higher MUNE values than the older men ($p = 0.024$), but MUNE values did not differ significantly across disciplines ($p = 0.679$) (**Table 2**). There was also a significant interaction between the effects of age and discipline in iMUNE ($p = 0.002$). Interaction contrasts revealed the age-related differences in iMUNE were significantly different in power athletes compared to age-related differences in controls (iMUNE contrast of differences: 12.52, 95% CI 4.57 to 20.47, $p = 0.002$) and age-related differences in endurance athletes (15.19, 95% CI 6.43 to 23.95, $p = 0.001$). There were no age-related differences between controls and endurance athletes (2.67, 95% CI −4.95 to 10.34, $p = 0.096$).

Intramuscular motor unit number estimates values were significantly larger in younger compared to older participants ($p < 0.001$) and pairwise comparisons showed this was consistent for all groups (controls $p < 0.001$; endurance $p = 0.002$; power $p < 0.001$). iMUNE values also differed across disciplines in the young ($p < 0.001$). Pairwise comparisons showed the iMUNE in young controls and young endurance were similar ($p = 0.296$), but young power had higher values than controls and endurance ($p = 0.006$ and $p < 0.001$, respectively). In older participants there was no significant effect of *discipline* on iMUNE ($p = 0.946$).

Athlete Motor Unit Potential Size

There was a significant interaction between the effects of age and discipline for MUP area (**Figure 5**, $p = 0.042$ for interaction). Interaction contrasts showed the age-related increase in MUP size was smaller in the controls than it was for the endurance athletes (234 mV.ms, 95% CI 12 to 456, $p = 0.038$) and power

TABLE 2 | Surface EMG measurements of the vastus lateralis.

	Young control (*n* = 15)	Young endurance (*n* = 8)	Young power (*n* = 11)	Old control (*n* = 22)	Old endurance (*n* = 18)	Old power (*n* = 11)	*P*-value for age × discipline interaction	*P*-value for age difference	*P*-value for discipline difference
CMAP (μV.ms)	98240 (20549)	92012 (14233)	98496 (24841)	65266 (15810)	74913 (19897)	70538 (10584)	0.263	**<0.001**	0.846
sMUP (μV.ms)	506 (234)	537 (201)	497 (223)	418 (145)	499 (178)	451 (215)	0.868	0.201	0.551
MUNE	233 (113)	199 (96)	219 (76)	168 (60)	168 (77)	184 (77)	0.687	**0.024**	0.679
MUs identified (number per person)	26 (12)	27 (12)	28 (11)	25 (9)	21 (10)	24 (11)	0.475	0.894	0.061

Interaction effects and main effects of age and discipline are shown in final columns. CMAP, compound muscle action potential; sMUP, surface motor unit potential; MUNE, motor unit number estimate. P-values for significant differences are shown in bold.

athletes (314 mV.ms 95% CI 89 to 539, $p = 0.013$). There was no significant difference in the age-related contrast estimates in MUP size between endurance and power athletes (97 mV.ms, 95% CI −147 to 337, $p = 0.292$; **Figure 5**).

In younger participants, MUP area was smaller in controls, endurance athletes and power athletes compared with their discipline-matched older counterparts (all $p < 0.001$). MUP area in the young did not differ significantly across disciplines ($p < 0.438$), but MUP area did differ significantly across disciplines in the old ($p < 0.001$). Pairwise comparisons showed that older controls had smaller MUPs than older endurance athletes ($p = 0.001$) and older power athletes ($p < 0.001$), while MUP area in older endurance and older power athletes did not differ ($p = 0.999$; **Figure 5**).

Masters Tennis Players

In the second part of the study, the BB of both arms (favored and non-favored) of masters tennis players (age: 74 ± 5.2 years) were compared. Of the 11 master tennis players included, 10 favored the right arm to hold the racquet and one favored the left arm. Muscle size assessed by thickness from ultrasound scanning did not differ significantly between arms, but strength was 7% greater in the favored arm ($p = 0.044$) (**Table 3**). The CMAP was 16% greater in the favored arm ($p = 0.039$), but there were no significant differences between arms in sMUP, MUNE or iMUNE values. MUPs recorded from the favored arm were around 20% larger than the non-favored arm ($p < 0.001$).

DISCUSSION

We describe here several novel observations: Firstly, MUP size was greater for athletic older men compared with younger athletes of the same discipline. MUP size was also greater for athletic old of both disciplines compared to non-athletic older men. Secondly, we show that older masters athletes have smaller CMAP and lower MUNE values for the VL than healthy younger men. MUNE values were similar for all groups of older men, regardless of their exercise discipline (control, endurance or power). Finally, the favored arm of masters tennis players had similar MUNE and iMUNE, but larger CMAP and MUP, compared with the non-favored arm. Taken together, these results suggest that for the VL and BB, long-term training does not prevent age-related decline in MU numbers, but may facilitate successful reinnervation of denervated fibers promoting an increase in MU size to preserve muscle function.

The athletes recruited to this study were nationally or internationally competitive. For example, the age-graded performance of young endurance athletes was 87%, equivalent to a 5 km run time of approximately 14:40 min. Their physical characteristics typified their competitive discipline; endurance athletes had the lowest body mass, fat and knee extensor MVC, while the young power athletes had relatively low body fat and the highest body mass, muscle mass and knee extensor MVC. The typical endurance characteristics were generally retained amongst older athletes, as we previously noted (Piasecki et al., 2018a).

The combination of type 2 fiber atrophy and loss of some type 1 and type 2 fibers determines the extent of muscle loss in older age (Lexell et al., 1988; McPhee et al., 2018; Wilkinson et al., 2018), which is associated with remodeling of MUs. A review of the available literature showed that healthy older limb muscles have approximately 40% fewer MUs and those that remain are around 30% larger than healthy young (Piasecki et al., 2016b). The extent of MU remodeling seems to influence overall muscle mass, as we previously showed that older sarcopenic individuals have approximately 50% fewer MUs compared with young and additionally, they fail to increase the average MU size (Piasecki et al., 2018c). One appealing hypothesis is that regular exercise throughout older age will help to retain MU numbers and by extension, muscle size and function. Some evidence to this effect was previously presented for the TA muscle (Power et al., 2010, 2016), but this was not replicated by our own past work (Piasecki et al., 2016a). Here, we show that VL CMAP and sMUP values were similar across all older groups (endurance, power and controls) and calculating MUNE values from these data suggests similar MU numbers across these older groups. In all cases, CMAP values were lower and sMUP values similar in old compared with young men.

A limitation of cross-sectional studies comparing young with old is that lifestyle and other secular changes might contribute to any observed differences. Akin to fiber number, it is probable that MU characteristics are also influenced by genetic factors (Degens and Korhonen, 2012). For these reasons, we included competitive masters tennis players to take advantage

TABLE 3 | Neuromuscular characteristics in the favored arm vs. the non-favored arm in masters tennis players.

	Favored arm *N* = 11	Non-favored arm *N* = 11	*P*-value
Muscle thickness (mm)	34.2 (4.8)	33.9 (4.7)	0.575
Elbow flexor MVC (N)	215 (68)	201 (70)	**0.044**
CMAP (μV)	13137 (4953)	11357 (4411)	**0.039**
sMUP (μV)	75.6 (26.9)	71.6 (42.1)	0.867
MUNE	194 (83)	163 (69)	0.188
iMUNE	31.0 (14.4)	31.6 (6.3)	0.979
MUP Area	1120 (709–1709)	934 (647–1430)	**<0.001**

mm, millimeters; CMAP, compound muscle action potential; sMUP, surface motor unit potential; MUNE, motor unit number estimate; iMUNE, intramuscular motor unit number estimate; MUP, motor unit potential. P-values for significant differences are shown in bold.

of discordant use of their arms to compare the localized effects of long-term exercise on MU characteristics. This study design minimizes influences of genetic and lifestyle factors. Here, MUNE values were similar for the BB of both arms, but the CMAP and the MUP size were larger in the BB of the favored arm.

Our results suggest that regular exercise does not preserve MU numbers into older age, but there are other possible benefits of training for MU plasticity. Regular exercise has been associated with possible improvements to fiber reinnervation in older age that would result in increased MU size and clustering of fibers of the same type, referred to as fiber type grouping (Mosole et al., 2014; Zampieri et al., 2015). Our work provides further direct evidence that MU size, estimated from the intramuscular MUP area, was larger in all groups of older compared to younger participants, which is in agreement with previous studies (Hourigan et al., 2015; Piasecki et al., 2016b). Importantly, age-related MUP size increases (assessed cross-sectionally) were greater for older endurance and power athletes than older controls (**Figure 4**), and were greater in the favored arm compared with the non-favored arm of masters tennis players (**Table 3**). Fiber reinnervation acts as a compensatory mechanism in response to MU loss (Gordon et al., 2004) and these results suggest lifelong exercise may facilitate this process. Although not a direct estimate of fiber number, the CMAP is an indicator of the amount of contractile material within the recording area of the surface electrode. This was larger in the favored compared to the non-favored arm of the tennis players, and although not significantly different, the VL CMAP was 8–15% larger in the older athletes compared to older controls. Thus, these data suggest the possibility of increased MU size in the master athletes and preserved muscle quality (Power et al., 2014; McPhee et al., 2018).

Regular intense exercise can preserve muscle mass and function in older age (Mckendry et al., 2018) by minimizing fiber atrophy and possibly by enhancing the rescue of denervated muscle fibers. It remains largely unknown how the local muscle milieu created by exercise leads to more successful rescue of orphaned fibers, but this will inevitably depend on facilitating successful axonal sprouting and remodeling of neuromuscular junctions (Deschenes, 2011; Gonzalez-Freire et al., 2014; Nishimune et al., 2014; Tintignac et al., 2015). However, caution should be shown when translating this evidence largely derived from animals into human aging (Jones et al., 2017).

Strengths and Limitations

A strength of this work was the relatively large sample size and use of decomposition-enhanced quantitative electromyography to compare MU characteristics between groups of older and younger men, and the athletes enrolled in this study were of a high caliber. A second strength of this work was that in addition to the direct comparisons across age and exercise disciplines, we also assessed masters tennis players to take advantage of different bi-lateral use of limbs to increase confidence that effects were related to the exercise habits rather than other lifestyle or genetic characteristics.

A limitation of the work was that it is not possible to directly measure the number of MUs in human volunteers. We have used the iMUNE method to provide an index based on the mean MUP size normalized to the muscle CSA, and the more established method of comparing the electrically evoked CMAP to an ensemble-averaged mean sMUP (MUNE) (Brown et al., 1988; Piasecki et al., 2018b). Although limited by the capture area of the recording electrodes, results from both techniques were generally consistent across age; older adults have lower MU number values than young, irrespective of training history (**Figure 4**). A second limitation is that all MUs were sampled during isometric contractions held at 25% MVC, which precludes definitive statements on MU remodeling assessed across the full MU pool where smaller/larger MUs would be recruited in accordance with the Henneman size principle (Henneman et al., 1965). Thirdly, muscle biopsies were not collected in the present study, so it is not possible to estimate the contribution that fiber atrophy has on the age-related loss of muscle mass across the three disciplines, and whilst not affecting the conclusions of this study, it is likely that fiber atrophy occurred in all old groups. Muscle biopsies could also be used in future studies to assess for possible connective tissue (McPhee et al., 2018) and fat accumulation (Hogrel et al., 2015) with aging. Their accumulation may affect tissue conduction to attenuate the measured MUP (or similarly sMUPs and CMAP for surface recordings), which would mean that the increased MUP size of older men in the present study is a conservative estimation of the actual effect of aging. Finally, although the athletes were nationally or internationally competitive within their disciplines, we do not have sufficient detail of their training programs to compare training regimens across groups.

CONCLUSION

Long-term athletic training does not prevent age-related decline of muscle size and the current findings suggest that MU numbers decrease even in athletic old. Long-term training into older age may facilitate more successful axonal sprouting and

reinnervation of denervated fibers to produce larger MUs with increased MUP size.

ETHICS STATEMENT

The study was conducted in accordance with the Declaration of Helsinki and approved by the University Research Ethics Committee and the National Research Ethics Service Committee Northwest (15/NW/0426). All participants provided written informed consent.

AUTHOR CONTRIBUTIONS

All authors contributed to the design of the work and analysis and interpretation of the data, and agreed to be accountable for all aspects of the work. MP, AI, JP, AS, and JM contributed to the acquisition of the data. MP and JM drafted the manuscript. AI, JP, HD, DS, AS, MR, and DJ provided comments and approved the final submission.

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Independent and Combined Effects of Antioxidant Supplementation and Circuit Resistance Training on Selected Adipokines in Postmenopausal Women

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We examined the effects of the independent and combined effects of Zataria Multiflora supplementation and circuit resistance training (CRT) on selected adipokines among postmenopausal women. Forty-eight postmenopausal women were divided into four groups: Exercise (EG, n = 12), Zataria Multiflora (ZMG, n = 12), exercise and Zataria Multiflora (ZMEG, n = 12), and control (CG, n = 12). Participants in experimental groups either performed CRT (3 sessions per week with intensity at 55% of one-repetition maximum) or supplemented with Zataria Multiflora (500 mg every day after breakfast with 100 ml of water), or their combination, for 8 weeks. Blood samples were collected at pre- and post-intervention for measuring selected adipokines, including visfatin, omentin-1, vaspin, FGF-21, adiponectin, leptin, and ghrelin. Our findings demonstrated that visfatin, vaspin, and leptin levels significantly decreased over the intervention period (all $p < 0.05$), with these values were lower in EG and ZMEG in comparison to CG at post-intervention (all $p < 0.05$). Visfatin and vaspin levels were also lower in ZMEG in comparison to EG at post-intervention (both $p < 0.05$). In contrast, omentin-1, ghrelin, adiponectin, and FGF21 significantly increased in EG and EMG (all $p < 0.05$) after CRT. These findings suggest that Zataria Multiflora supplementation by itself has little effect on measured adipokines; however, its combination with CRT produced noticeable effects on circulating levels of these adipokines, even more than CRT alone. Consequently, a combination of CRT and Zataria Multiflora supplementation may represent a potentially beneficial non-pharmacologic intervention on some selected adipokines in postmenopausal women.

Keywords: postmenopausal women, Zataria Multiflora, adipokine, visfatin, vaspin, leptin, inflammation, metabolic syndrome

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INTRODUCTION

Menopausal transition is associated with weight gain, possibly by an increase in visceral and total body fat that usually originates form reduction in total energy expenditure as a consequence of physical inactivity, coupled with depression, age-induced sarcopenia, and lower basal metabolic rate (Davis et al., 2012; Al-Safi and Polotsky, 2015). It has also been reported that menopausal transition induces variations in the circulating levels of adipokines; however, it is unclear as to whether those changes could be explained by obesity (Sowers et al., 2008). Therefore, identifying and mapping strategies (i.e., exercise and healthy diet) to prevent and/or minimize gaining weight in postmenopausal individuals is of profound importance.

Adipokines regulates numerous biological processes in systemic organs, such as brain, liver, skeletal muscle, heart, and endocrine glands (Lehr et al., 2012). Deregulated production or secretion of these adipokines is reported with obesity, which could subsequently contribute in appetite and satiety disturbances, adipose tissue distribution, insulin sensitivity and secretion abnormalities, endothelial function, angiogenesis, inflammation, blood pressure, hemostasis, and osteoarticular functions and reproduction dysfunctions (Blüher, 2014). This deregulation of adipokines is exemplified by the direct and positive association between insulin resistance, diabetes, and obesity-related diseases with some adipokines, like leptin, vaspin, and visfatin, and an inverse association between those diseases and other adipokines, like omentin-1, adiponectin, and FGF-21 (Blüher, 2014; Fève et al., 2016). Consequently, any modulation, by exercise, supplementation, or their combination, on these substances may provide favorable prospects of mitigating obesity-related morbidities.

Amongst various forms of resistance training, circuit resistance training (CRT) is reported to improve maximum oxygen consumption, functional capacity, and body composition and strength (Camargo et al., 2008; Bocalini et al., 2012). While CRT is suggested as a time-efficient training modality that can elicit health benefits in various healthy and clinical populations, including postmenopausal individuals (Brentano et al., 2008; Williams et al., 2013), most clinical investigations have utilized aerobic exercise training (i.e., treadmill and cycling) as a strategy to induce desired changes in body composition and metabolic syndrome markers (Pearsall et al., 2014; Wiklund et al., 2014; Guadalupe-Grau et al., 2018; Mora-Rodriguez et al., 2018). It is reported, for example, that aerobic exercise training, alone or combined with hypocaloric diet, improve symptoms of the metabolic syndromes, presumably through changing the levels of inflammatory adipokines (You and Nicklas, 2008). Further, a newly released research revealed that 6 months of aerobic interval training improve the capacity for fat oxidation during exercise and enhance the maximal oxygen consumption, coupled with skeletal muscle improvement in mitochondrial enzyme activity (Guadalupe-Grau et al., 2018). Taken together, the effects of CRT on health-related parameters, such as body composition and metabolic syndrome markers, adipokines in particular, within clinical populations, especially postmenopausal, remain unanswered and needs to be investigated.

Herbal remedies, also known as herbal medicine, has for decades gained a great deal of interest among researchers all over the globe as a healthier alternative to medication in order to either prevent, alleviate, or minimize diseases outcomes (Ekor, 2014). Zatraia Multiflora (Aavishan-Shirazi) is one of those herbs which is popular for its positive impacts (e.g., antibacterial, antifungal, analgesic, and antioxidant- and immune-regulatory effects) on body function (Boskabady and Gholami Mhtaj, 2014). A recent study, in animal, found increased levels of adipokines following Zataria Multiflora supplementation (Mohammadi et al., 2014). These authors suggested the increased gamma peroxisome proliferator-activated receptor (PPAR) protein as a potential mechanism for this response. It is, therefore, plausible that Zataria Multiflora may have some effects on metabolic syndrome markers including adipokines. This underlines the needs for human-based research investigating the potential modulatory effects of Zataria Multiflora on adipokines, especially in postmenopausal individuals.

To the best of our knowledge, no study to date has examined the independent and/or combined effects of Zataria Multiflora supplementation either on selected adipokines or in postmenopausal individuals. Thus, the present study was conducted to provide some preliminary results on the effects of Zataria Multiflora supplementation and exercise training on some selected adipokines within postmenopausal.

MATERIALS AND METHODS

Experimental Design and Study Population

The experimental design of the present study is illustrated in **Figure 1**. This study included 48 postmenopausal participants. Participants were matched in terms of age, body weight, and body mass index and then divided into four groups: exercise (EG, n = 12), Zataria Multiflora (ZMG, n = 12), exercise and Zataria Multiflora (EZMG, n = 12), and control (CG, n = 12). Inclusion criteria for participation were: (1) 6 months or more pass menopause, (2) no prescribed drugs consumption before exercise session and/or alcohol addiction, (3) no history of regular exercise for at least 6 months, (4) no history of renal, hepatic, cardiovascular disease, diabetes and/or any physical injury or problem as a physical activity obstacle. Postmenopausal status was confirmed by postmenopausal levels of serum estradiol (<120 pmol/l) and follicle-stimulating hormone (FSH > 30 IU/l) and through examination by a gynecologist. Participants in experimental groups either performed CRT or supplemented with Zataria Multiflora, or their combination, for 8 weeks. Blood samples were collected at pre- and post-intervention for measuring selected adipokines. It should be noted that although no side effects have been reported with the dosage used in the present study, all participants were informed for any potential adverse effects of consuming herbal supplements prior to the study (Ghanbari-Niaki et al., 2018; Tayebi et al., 2018, 2019). Moreover, throughout the study participants had free access to a physician whenever they needed it.

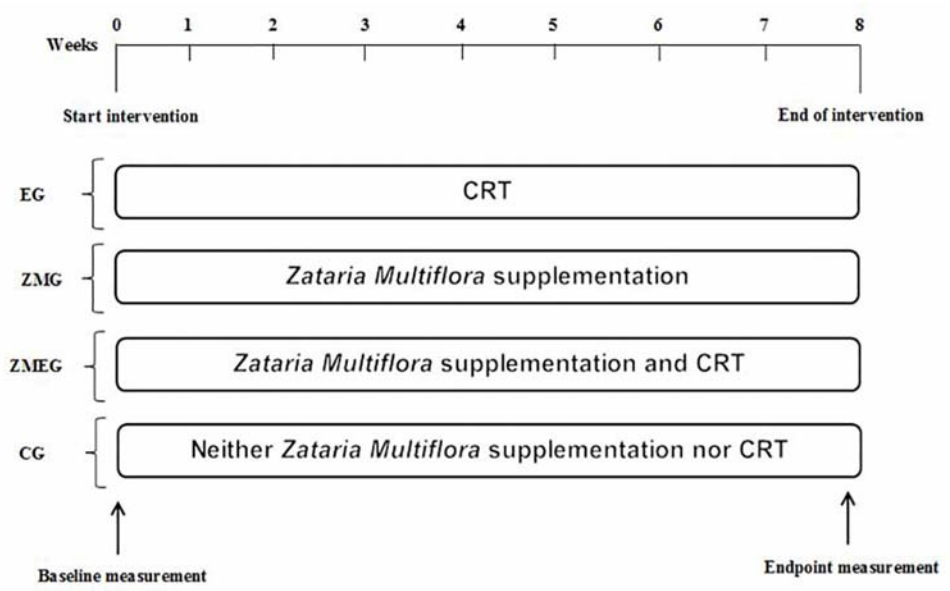

FIGURE 1 | Experimental design of 8 weeks of CRT and *Zataria Multifloria* supplementation on adipokines response in postmenopausal. *CRT*, circuit exercise training; *EG*, exercise group; *ZMG*, *Zataria Multifloria* group; ZMEG, *Zataria Multifloria* and exercise group*; CG,* control group.

Finally, prior to the study beginning, all participants were accessed for any health issues (e.g., high blood pressure), which precluded their participation in the study. Therefore, participants were healthy which minimized the magnitude of potential unanticipated side effects occurring. This study was conducted according to the ethical principles of the Declaration of Helsinki, with informed written consent forms were signed by all participants.

Zataria Multiflora Collection, Extraction, and GCMS

Zataria Multiflora leaves were collected in March. The leaves were cleaned, dried in the shade for 10 days, dried in an oven for 48 h at a temperature of 32°C and then powdered. 50 g of powdered sample was extracted using the water distillation method in a Clevenger apparatus at the boiling point for 3 h. The extract was filtered, dried on anhydrous sodium sulfate, transferred into a glass container (closed the lid) and stored at 4°C. The essential oil yield was calculated as dry essential oil volume divided by the initial dry powder weight multiplied by 100. In this fashion the yield calculated was 3% (Saei-Dehkordi et al., 2010). Agilent Gas Chromatography/Mas Spectrophotometry (GC-MS, Agilent, USA GC 7890A, MS 5975C, Agilant, United States) was used in order to identify the most volatile components of the powder of Zataria Multiflora. The GC was equipped with a HP-5 column (30 m length × 0.25 mm i.d., film thickness 0.25 μm coupled with an Agilent 5975 mass spectrometer). The column temperature was programmed at 50°C as an initial temperature, holding for 5 min, with 3°C increases per minute to the temperature of 240°C, followed by a temperature enhancement of 15°C per minute up to 300°C, holding at the mentioned temperature for 3 min. Injector port temperature was 290°C and helium used as carrier gas at a flow rate of 1.5 ml/min (Saei-Dehkordi et al., 2010). Afterward, the compounds in Zataria Multiflora essential oil were identified using fragmentation pattern in the database of wiley7n.l and NIST08 and also using the retention time in the chromatography column (Saei-Dehkordi et al., 2010). The ratio of peak area for each component to the total area was subsequently calculated. These results are shown in **Table 1**.

Zataria Multiflora Supplementation

A pilot study had been conducted to determine the dosage to be used in the present study. Briefly, a total of 20 participants were

TABLE 1 | The main components of the Zataria Multiflora as measured by GC-MS analysis.

Components	The area under the Peak (%)	Retention time (min)
Thymol	35:09	26.8
Carvacrol	36:5	22.9
p-cymene	22:03	7.7
γ-terpinene	23:55	6.8
α-pinene	16:53	3.2
β-caryophyllene	41:36	3
Carvacrolmethyl ether	33:26	2.4
α-terpinene	21:12	2.2
Spathulenol	48:36	2
Linalool	25:59	1.8
β-myrcene	20:00	1.5
	Total	80.3

supplemented with Zataria Multiflora using three previously recommended dosages (100, 200, and 500 mg) for 1 month, followed by antioxidant and insulin resistance measurements at the end of the supplementation. The findings indicated that 500 mg consumption was the most effective dosage to significantly increasing or decreasing antioxidant activity and insulin resistance, respectively. Additionally, it should be noted that previous studies have reported no major side effects of 500 mg dosage (Ghanbari-Niaki et al., 2018; Tayebi et al., 2018, 2019). Therefore, a total of 500 mg of dry Zataria Multiflora leaves powder was placed in capsules. The ZMG and EZMG groups received a capsule (500 mg) of Zataria Multiflora every day after breakfast with 100 ml of water. The EG and CG groups consumed placebo capsules (500 mg wheat flour) after breakfast with 100 ml of water for a total of 8 weeks. The participants consumed the capsules on a daily basis in the presence of investigators.

Exercise Training Protocol

Participants were familiarized with the environment and CRT movements for 1 week and then 1 repetition maximum (1-RM) for each of the given exercises was determined. The 1-RM for each exercise movement was calculated using Brzezinski equation (Ghanbari-Niaki et al., 2015). Training sessions were delivered using CRT format with alternation between upper-body and lower-body movements as well as multi-joint movements at the beginning of the movements (Ghanbari-Niaki et al., 2015). The exercises included: (1) Squat, (2) Chest press, (3) Leg press, (4) Standing Military Press, (5) Knee extension, (6) Seated cable rowing, (7) Knee Curl, (8) Biceps curl, (9) standing calf raise, (10) Triceps press, (11) Back extension, and (12) Abdominal crunch. Participants in the EG and EZMG groups performed movements at 55% of 1-RM for 8 weeks (3 sessions per week). It has been suggested to achieve an optimal cardiovascular health and also to reduce the risk of cardiovascular diseases, individuals should perform exercise training, in this case resistance training, with 40–55% of 1RM (American College of Sports Medicine [ACSM] , 2017). Hence, in the present study we chose 55% of 1RM since it has been reported that this intensity can result in a greater amount of fat oxidation (American College of Sports Medicine [ACSM] , 2017). Each exercise session included a 5 min warm-up and then followed by the 12 prescribed exercises, with duration of approximately 30 s at each exercise station. The number of repetitions at each station was recorded for the participants. In each session, two sets (turns) of 12 exercises were carried out such that between each set, there was a 3 min active rest period (Ghanbari-Niaki et al., 2015).

Blood Sampling and Adipokines Measurements

Blood samples were collected at pre- and post-intervention from an antecubital vein in sitting position. Participants were required to meet the following criteria for blood sampling: (1) no exercise other than the prescribed exercise regime of the study at least 72 h before blood sampling, and (2) No drink or consume specific beverage or food such as coffee, dark tea, banana, and cereal 24 h before blood sampling. Blood samples were placed into EDTA (plasma) and sterile (serum) tubes. All samples were centrifuged and then were stored at −70°C until analysis. Following adipokines were measured using commercially available assay kits: (1) plasma visfatin (Cat. No. V0523EK, enzyme-linked immunosorbent [ELISA] assay kit, AdipoGen, Seoul, Korea); (2) serum omentin-1 (Cat. No. APO-54N-034, sandwich ELISA, ELISA kit, Apotech Corp., Switzerland); (3) plasma vaspin (Cat. No. CSB-E09771h, ELISA kit, Cusabio Biotech, Wuhan, China); (4) plasma FGF-21 (Cat. No. RD191108200R, sandwich ELISA kit, Biovendor, Heidelberg, Germany); (5) plasma adiponectin (Cat. No. RD191023100, sandwich ELISA kit, Biovendor, Heidelberg, Germany); (6) plasma leptin (Cat. No. RD191001100, sandwich ELISA kit, Biovendor, Heidelberg, Germany); and (7) plasma ghrelin (Cat. No. 171B7104M, ELISA kit - Bio-Plex 200 System, Bio-Rad Laboratories, Gurgaon, India). The Elisa machine used in the current project was made in Biohit Company in Finland. It was programmed to CV = 7.59 in terms of qualitative control. Moreover, in order to boost the measurement accuracy; all the tests were double checked.

Statistical Analysis

All data are expressed as mean ± SD. The distribution of each variable was examined with the Shapiro–Wilk normality test. The overall effect of exercise and Zataria Multiflora supplementation was determined on selected adipokines prior to and post 8 weeks CRT using a mixed-model repeated-measures analysis of variance (group × time) with Bonferroni *post hoc* test. Statistical significance was set at $p < 0.05$. All data analysis was carried out using Statistical Package for the Social Sciences (SPSS version 23 for Windows).

RESULTS

Physical Characteristics

Baseline physical characteristics of the participants are shown in **Table 2**. All groups were similar in terms of age, height, body weight, BMI ($p > 0.05$).

Selected Adipokines Levels Prior to and Post 8 Weeks CR

Visfatin

There was a significant interaction between time and group for visfatin (**Figure 2**) [$F(3,44) = 13.5$, $p = 0.000$, $\eta^2 = 0.48$].

TABLE 2 | Anthropometric characteristics of study participants.

	CG	ZMG	EG	ZMEG
Age (years)	56 ± 5	54 ± 4	58. ± 5	54 ± 6
Height (cm)	156.7 ± 3.5	160.9 ± 4.1	158.7 ± 3.8	159.2 ± 4.7
Body weight (kg)	68.7 ± 13.3	66.4 ± 10.9	67.8 ± 13.01	70.1 ± 10.8
BMI (kg/m^2)	28.2 ± 1.9	25.9 ± 2.5	27.2 ± 1.8	27.8 ± 2.0

CG, Control Group; ZMG, Zataria Multiflora group; EG, Exercise group; ZMEG, Exercise and Zataria Multiflora; BMI, Body mass index.

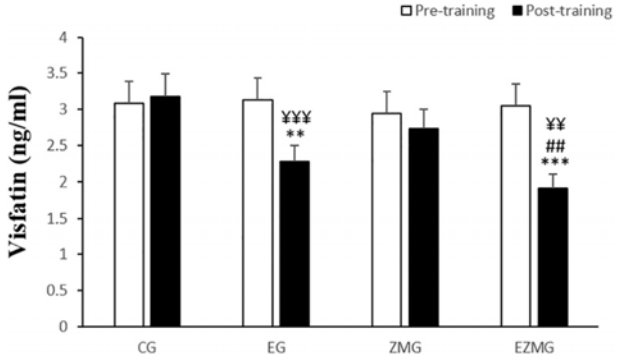

FIGURE 2 | Visfatin levels in pre and post training. *CG*, control group; *EG*, exercise group; *ZMG*, Zataria Multiflora group; E*ZMG*, Exercise and Zataria Multiflora group. Data are expressed as means ± SD. $^{**}P < 0.01$, $^{***}P < 0.001$ versus post-test in CG group. $^{\#\#}P < 0.01$ versus post-test in ZMG group. $^{¥¥}P < 0.01$, $^{¥¥¥}P < 0.001$ versus pre-test in same group.

Visfatin levels were significantly different between groups at post-intervention [$F(3,44) = 12.5$, $p = 0.000$, $\eta^2 = 0.46$], with significant lower values in EG (mean = 2.27, *SD* = 0.49, $p = 0.001$) and ZMEG (mean = 1.91, *SD* = 0.58, $p = 0.000$) in comparison with CG (mean = 3.17, *SD* = 0.5). A significant lower value was also noted for ZMEG (mean = 1.91, *SD* = 0.58) in comparison with EG (mean = 2.27, *SD* = 0.49, $p = 0.003$) at post-intervention. There were significant differences over the time for visfatin in EG [$F(1,44) = 30.4$, $p = 0.000$, $\eta^2 = 0.4$] and ZMEG [$F(1,44) = 54.3$, $p = 0.000$, $\eta^2 = 0.55$], with lower values at post-intervention (EG = 2.27, $p = 0.000$, ZMEG = 1.91, $p = 0.000$) in comparison with baseline (EG = 3.12, *SD* = 0.6, ZMEG = 3.05, *SD* = 0.8).

Vaspin

There was a significant interaction between time and group for vaspin (**Figure 3**) [$F(3,44) = 17.9$, $p = 0.000$, $\eta^2 = 0.55$]. Vaspin levels were significantly different between groups at post-intervention [$F(3,44) = 13.2$, $p = 0.000$, $\eta^2 = 0.47$], with significant lower values in EG (mean = 414.7, *SD* = 7.5, $p = 0.01$) and ZMEG (mean = 412.4, *SD* = 7.5, $p = 0.005$) in comparison with CG (mean = 450.6, *SD* = 7.5). Vaspin levels were also lower at post-intervention in EG (mean = 414.7, *SD* = 7.5, $p = 0.000$) and ZMEG (mean = 412.4, *SD* = 7.5, $p = 0.000$) in comparison with ZMG (mean = 468.5, *SD* = 7.5), and in ZMEG (mean = 412.4, *SD* = 7.5) in comparison with EG (mean = 414.7, *SD* = 7.5). There were significant differences over the time for vaspin in EG [$F(1,44) = 38.9$, $p = 0.000$, $\eta^2 = 0.47$], ZMG [$F(1,44) = 4.7$, $p = 0.034$, $\eta^2 = 0.98$], and ZMEG [$F(1,44) = 32.9$, $p = 0.000$, $\eta^2 = 0.42$], with lower values at post-intervention in EG (mean = 414.7, *SD* = 7.5, $p = 0.000$) and ZMEG (mean = 412.4, *SD* = 7.5, $p = 0.000$), and higher values in ZMG (mean = 468.5, *SD* = 7.5, $p = 0.34$), all compared to baseline (EG = 457.6, *SD* = 35.7, ZMEG = 451.8, *SD* = 31.7, ZMG = 453.5, *SD* = 35.1).

Omentin-1

There was a significant interaction between time and group for omentin-1 (**Figure 4**) [$F(3,44) = 5.14$, $p = 0.004$, $\eta^2 = 0.26$]. Omentin-1 levels were significantly different between groups at post-intervention [$F(3,44) = 9.2$, $p = 0.000$, $\eta^2 = 0.38$], with significant higher values in EG (mean = 14.5, *SD* = 0.49, $p = 0.048$) and ZMEG (mean = 16.1, *SD* = 0.49, $p = 0.000$) in comparison with CG (mean = 12.5, *SD* = 0.49). Omentin-1 levels were also higher in ZMEG (mean = 16.1, *SD* = 0.49, $p = 0.003$) in comparison ZMG (mean = 13.4, *SD* = 0.49). There were significant differences over the time for omentin-1 in EG [$F(1,44) = 11.4$, $p = 0.002$, $\eta^2 = 0.2$] and ZMEG [$F(1,44) = 19.8$, $p = 0.000$, $\eta^2 = 0.31$], with higher values at post-intervention in EG (mean = 14.5, *SD* = 0.49, $p = 0.002$) and ZMEG (mean = 16.1, *SD* = 0.49, $p = 0.000$) in comparison with baseline (EG = 12.2, *SD* = 1.8, ZMEG = 13.05, *SD* = 3.5).

Ghrelin

No significant interaction [$F(3,44) = 0.8$, $p = 0.46$, $\eta^2 = 0.05$] and group [$F(3,44) = 0.7$, $p = 0.54$, $\eta^2 = 0.4$] effects were noted for ghrelin (**Figure 5**). However, a significant main effect for time [$F(1,44) = 8.1$, $p = 0.001$, $\eta^2 = 0.15$] was noted for ghrelin, with higher values at post-intervention in comparison with baseline.

Adiponectin

There was a significant interaction between time and group for adiponectin (**Figure 6**) [$F(3,44) = 21.4$, $p = 0.000$, $\eta^2 = 0.59$]. Adiponectin levels were significantly different between groups at post-intervention [$F(3,44) = 20.5$, $p = 0.000$, $\eta^2 = 0.58$], with significant higher values in EG (mean = 13.8, *SD* = 0.23, $p = 0.002$), ZMG (mean = 13.7, *SD* = 0.23, $p = 0.006$), and ZMEG (mean = 15.1, *SD* = 0.23, $p = 0.000$) in comparison with CG (mean = 12.5, *SD* = 0.23). Adiponectin levels were also higher at post-intervention in ZMEG (mean = 15.1, *SD* = 0.23) in comparison with ZMG (mean = 13.7, *SD* = 0.23, $p = 0.001$) and EG (mean = 13.8, *SD* = 0.23, $p = 0.002$). There were significant differences over the time for adiponectin in EG [$F(1,44) = 26.2$, $p = 0.000$, $\eta^2 = 0.37$], ZMG [$F(1,44) = 13.9$, $p = 0.001$, $\eta^2 = 0.23$], and ZMEG [$F(1,44) = 102.6$, $p = 0.000$, $\eta^2 = 0.7$], with higher values at post-intervention in EG (mean = 13.8, *SD* = 0.23, $p = 0.000$), ZMG (mean = 13.7, *SD* = 0.23, $p = 0.001$), and ZMEG (mean = 15.1, *SD* = 0.23, $p = 0.000$) in comparison to baseline (EG = 12.8, *SD* = 1, ZMEG = 13.1, *SD* = 0.6, ZMG = 13.01, *SD* = 0.7).

Leptin

There was a significant interaction between time and group for leptin (**Figure 7**) [$F(3,44) = 25.9$, $p = 0.000$, $\eta^2 = 0.63$]. Leptin levels were significantly different between groups at post-intervention [$F(3,44) = 10.1$, $p = 0.000$, $\eta^2 = 0.4$], with significant lower values in EG (mean = 19, *SD* = 0.49, $p = 0.006$) and ZMEG (mean = 17.7, *SD* = 0.49, $p = 0.000$) in comparison with CG (mean = 21.4, *SD* = 0.49). Leptin levels were also lower at post-intervention in ZMEG (mean = 17.7, *SD* = 0.49, $p = 0.04$) in comparison with ZMG (mean = 19.6, *SD* = 0.49). There were significant differences over the time for leptin in EG [$F(1,44) = 23.3$, $p = 0.000$, $\eta^2 = 0.34$], ZMG [$F(1,44) = 17.4$, $p = 0.000$, $\eta^2 = 0.28$], ZMEG [$F(1,44) = 88.6$, $p = 0.000$, $\eta^2 = 0.66$], and CG [$F(1.44) = 9$, $p = 004$, $\eta^2 = 0.17$]. with lower values at post-intervention in EG (mean = 13.8, *SD* = 0.23, $p = 0.000$), ZMG (mean = 13.7, *SD* = 0.23, $p = 0.001$), and ZMEG (mean = 15.1,

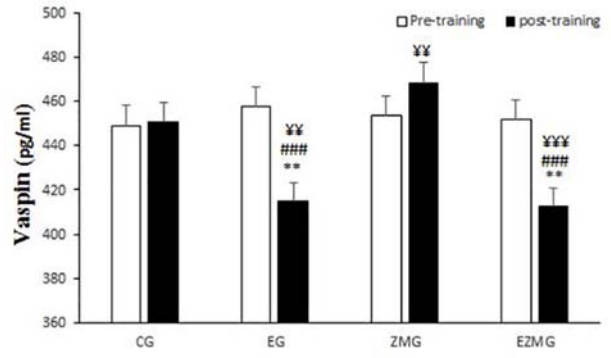

FIGURE 3 | Vaspin levels in pre and post training. *CG*, control group; *EG*, exercise group; *ZMG*, Zataria Multiflora group; E*ZMG*, Exercise and Zataria Multiflora group. Data are expressed as means ± SD. $^{**}P < 0.01$ versus post-test in CG group. $^{\#\#\#}P < 0.001$ versus post-test in ZMG group. $^{¥¥}P < 0.01$, $^{¥¥¥}P < 0.001$ versus pre-test in same group.

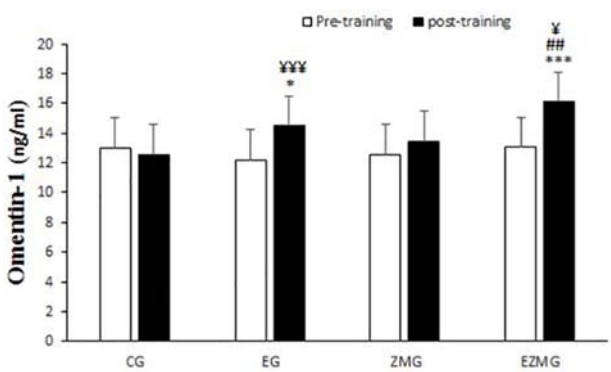

FIGURE 4 | Omentin-1 levels in pre and post training. *CG*, control group; *EG*, exercise group; *ZMG*, Zataria Multiflora group; E*ZMG*, Exercise and Zataria Multiflora group. Data are expressed as means ± SD. $^{*}P < 0.05$, $^{***}P < 0.01$ versus post-test in CG group. $^{\#\#}P < 0.01$ versus post-test in ZMG group. $^{¥}P < 0.05$, $^{¥¥¥}P < 0.001$ versus pre-test in same group.

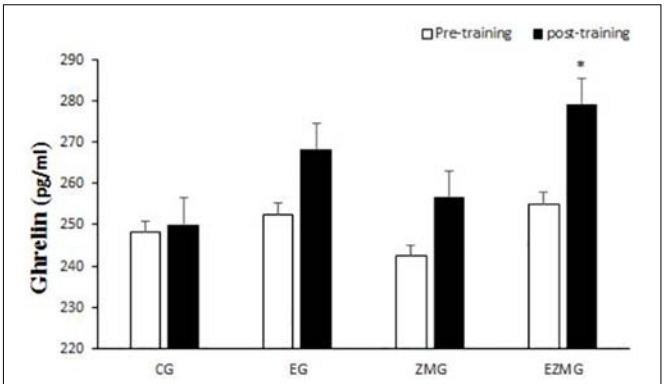

FIGURE 5 | Ghrelin levels in pre and post training. *CG*, control group; *EG*, exercise group; *ZMG*, Zataria Multiflora group; E*ZMG*, Exercise and Zataria Multiflora group. Data are expressed as means ± SD. $^{*}P < 0.05$versus post-test in CG group.

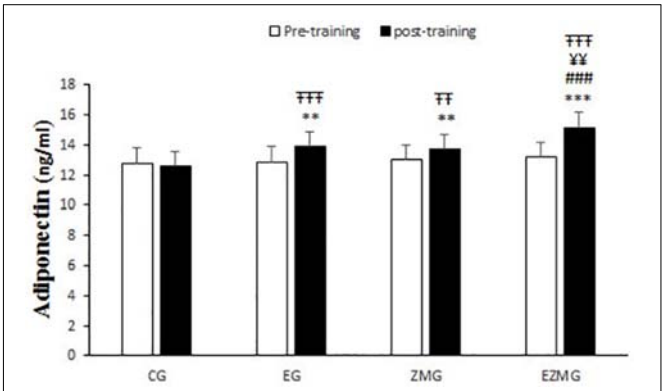

FIGURE 6 | Adiponectin levels in pre and post training. *CG*, control group; *EG*, exercise group; *ZMG*, Zataria Multiflora group; E*ZMG*, Exercise and Zataria Multiflora group. Data are expressed as means ± SD.$^{**}P < 0.01$, $^{***}P < 0.001$ versus post-test in CG group. $^{\#\#\#}P < 0.001$ versus post-test in ZMG group. $^{¥¥}P < 0.01$ versus post-test in EG group. $^{TT}P < 0.01$, $^{TTT}P < 0.001$ versus pre-test in same group.

SD = 0.23, $p = 0.000$), and higher values in CG (mean = 21.4, *SD* = 0.4), all compared to baseline (EG = 20.6, *SD* = 0.4, ZMEG = 20.9, *SD* = 0.4, ZMG = 21, *SD* = 0.4, CG = 20.4, *SD* = 0.4).

FGF21

There was a significant interaction between time and group for FGF21 (**Figure 8**) [$F(3,44) = 6.1$, $p = 0.001$, $\eta^2 = 0.29$]. FGF21levels were significantly different between groups at post-intervention [$F(3,44) = 14.1$, $p = 0.000$, $\eta^2 = 0.49$], with significant higher values in EG (mean = 281.6, *SD* = 5.5, $p = 0.004$) and ZMEG (mean = 300.9, *SD* = 5.5, $p = 0.000$) in comparison with CG (mean = 252.7, *SD* = 5.5). FGF21levels were also higher at post-intervention in ZMEG (mean = 300.9, *SD* = 5.5, $p = 0.000$) in comparison with ZMG (mean = 265.7, *SD* = 5.5). There were significant differences over the time for FGF21in EG [$F(1,44) = 12.5$, $p = 0.001$, $\eta^2 = 0.22$] and ZMEG [$F(1,44) = 30.7$, $p = 0.000$, $\eta^2 = 0.41$], with higher values at post-intervention in EG (mean = 281.6, *SD* = 5.5, $p = 0.001$) and ZMEG (mean = 300.9, *SD* = 5.5, $p = 0.000$) in comparison with baseline (EG = 254.2, *SD* = 5.6, ZMEG = 258.1, *SD* = 5.6).

DISCUSSION

The beneficial effects of exercise-induced changes on select circulating adipokines in diverse populations have been well investigated (Sakurai et al., 2013). However, there does not appear to have been any study to date that has examined the combined effect of exercise training (i.e., resistance [CRT]) and herbal supplements (i.e., Zataria Multiflora) on circulating adipokines in postmenopausal women. To the best of our knowledge, this is the first study to report on the independent and combined effects of CRT and Zataria Multiflora on selected circulating adipokines in postmenopausal women. The present study tested the hypothesis that 8 weeks Zataria Multiflora supplementation along with CRT would have additional positive effects more than alone

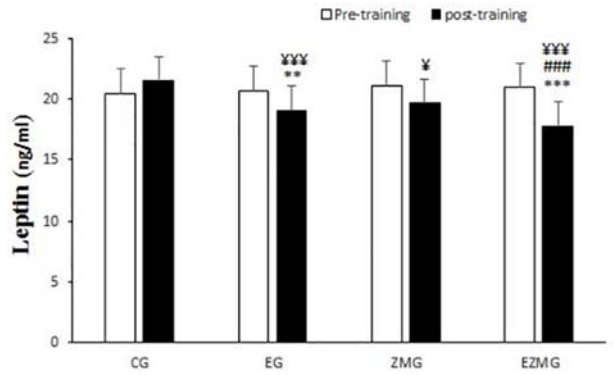

FIGURE 7 | Leptin levels in pre and post training. *CG*, control group; *EG*, exercise group; *ZMG*, Zataria Multiflora group; E*ZMG*, Exercise and Zataria Multiflora group. Data are expressed as means ± SD.$^{**}P < 0.01$, $^{***}P < 0.001$ versus post-test in CG group. $^{###}P < 0.001$ versus post-test in ZMG group. $^{¥}P < 0.05$, $^{¥¥¥}P < 0.001$ versus pre-test in same group.

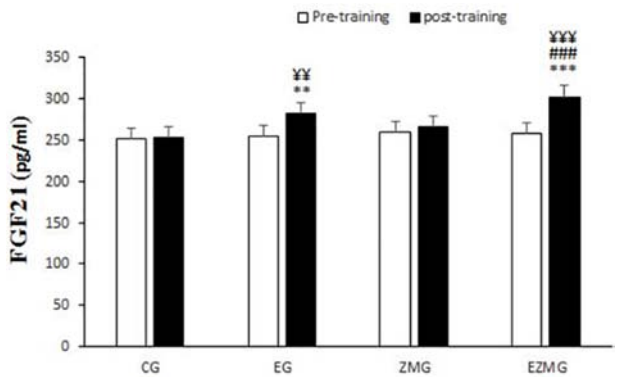

FIGURE 8 | FGF21 levels in pre and post training. *CG*, control group; *EG*, exercise group; *ZMG*, Zataria Multiflora group; E*ZMG*, Exercise and Zataria Multiflora group. Data are expressed as means ± SD.$^{**}P < 0.01$, $^{***}P < 0.001$ versus post-test in CG group. $^{###}P < 0.001$ versus post-test in ZMG group. $^{¥¥}P < 0.01$, $^{¥¥¥}P < 0.001$ versus pre-test in same group.

on circulating adipokines within postmenopausal women. The intervention was well controlled and the participants reported no side effects (e.g., nausea, weariness, headache, dizziness, and stomach problems or sleeplessness, etc.) during and/or following 8 weeks Zataria Multiflora supplementation. Our main findings in the present study were that the CRT and CRT plus Zataria Multiflora supplementation had more individual effects on most adipokines. However, Zataria Multiflora supplementation has little effect alone, indicating CRT as a critical element in the interventions we implemented.

Visfatin

While plenty of studies have been conducted on visfatin and its role in metabolism, most of them suggest that visfatin is a highly expressed adipokine in visceral adipose tissue (VAT), which mediates glucose homeostasis (Seo et al., 2011). Moreover, it makes a nutrient-sensing mechanism, modulate B-cell function and finally prevent diabetes (Haus et al., 2009). Our results showed that 8 weeks of CRT was the single greatest reason for the significant decrease of visfatin plasma levels in postmenopausal women. Zataria Multiflora did not show an effect on visfatin without being combined with CRT exercise. Despite antioxidant and anti-inflammatory effect of Zataria Multiflora (Boskabady and Gholami Mhtaj, 2014) significant effect on visfatin in postmenopausal women was not observed. This study was the first study about the effect of Zataria Multiflora supplementation on visfatin. Therefore, it seems since visfatin is highly expressed in adipose tissue (Seo et al., 2011) and in the postmenopausal stage body fat usually increases (Goodpaster et al., 2005), the Zataria Multiflora could not modulate visfatin level by itself during or the duration of the study (8 weeks period) was not sufficient. On the other hand, a combination of supplementation and CRT was more effective than exercise alone.

Vaspin and Omentin-1

Among the known adipokines, vaspin and omentin-1 play a key role in metabolism. In fact, there is a specific relationship among the body mass index (BMI), insulin sensitivity and glucose tolerance with the expression of vaspin *in vivo* (Heiker, 2014). Omentin-1 has an undeniable role in the enhancement of insulin-stimulated glucose uptake (Tan et al., 2008) or triggering Akt signaling in the lack or presence of insulin (Schäffler et al., 2005). Results from this study showed, as visfatin, CRT is the main reason for the change in the level of vaspin as well as omentin-1 plasma after 8 weeks period. In addition, Zataria Multiflora could increase vaspin plasma levels significantly without exercise. Thymol and carvacrol are the most important ingredients of Zataria Multiflora, which would bring some advantages in phase 1 metabolism, fatty acid oxidation and immune response (Hashemipour et al., 2013). It seems that the defined dosage of Zataria Multiflora used in the present study can modify vaspin plasma level regardless of CRT and can be a suitable supplement to decrease inflammation associated with this biomarker. To that end, vaspin has been reported to inhibit NF-KB and the expression of TNF α- and interleukin-1 induced adhesion molecules and to protect vascular endothelial cells. Various studies have shown the effects of insulin resistance and anti-inflammatory properties of vaspin (Liu et al., 2014). It has also been suggested that vaspin can increase the capacity of insulin absorption and insulin sensitivity, and ultimately affect glucose metabolism by regulating the proteolysis cascades and the gene expression level of the metabolic signaling pathways in the target tissues of the insulin (Liu et al., 2018). Additionally, vaspin has anti-inflammatory effects through inhibiting the expression of pre-inflammatory cytokines such as leptin, resistin and TNF-α. Furthermore, vaspin can reduce the inflammatory responses of smooth muscle cells caused by TNF-α by inhibiting ROS/PKC/NF-KB signaling (Liu et al., 2018). Zataria Multiflura likewise has potent antioxidants such as carvacrol and thymol that can reduce insulin resistance and inflammation by increasing vaspin levels. On the other hand, exercise training can reduce insulin resistance through GLUT-4 displacement, increased HDL and decreased triglyceride, LDL, and cholesterol, increased adiponectin and omentin 1 and reduced TNF-alpha reduced

(Halverstadt et al., 2007; Jorge et al., 2011). In the present study, the administration of exercise training, independent of vaspin, and through these mechanisms just noted reduced insulin resistance and required no increase in vaspin.

Gherlin, Leptin, and Adiponectin

The postmenopausal life stage is accompanied with the decline of estrogen levels in women, which leads to higher visceral fat percent (Mosca et al., 2007). Irregular hemostasis may make weight loss difficult even with implying some rigorous weight loss programs (Mosca et al., 2007). Adipose tissue is an important endocrine organ, which regulate different metabolic processes (Fève et al., 2016). Peptides including ghrelin, leptin and adiponectin play an important role in changing body composition, mediating hemostasis and inflammation (Blüher, 2014). In a closer view, ghrelin, known as a hunger signal, triggers meal initiation, and usually its level should be increased during weight loss (Kotidis et al., 2006). Also, there is evidence of its role in gastrointestinal motility, glucose metabolism and anti-inflammation (Tack et al., 2006). On the other hand, leptin has the opposite effect of ghrelin; i.e., its levels fall during periods of starvation (Hellström et al., 2004), and it has a positive correlation with systematic inflammation or even atherosclerotic diseases (Myers et al., 2008). Adiponectin has a different pattern compared to two others and is not affected by food intake during a day (Small and Bloom, 2004). Its role in insulin sensation and setting up anti-inflammatory cascade has been completely proved (Brochu-Gaudreau et al., 2010). Dietary-induced weight loss is accompanied by the rise of ghrelin plasma concentration, while leptin and adiponectin, usually decreases and increases during weight loss period respectively (Kotidis et al., 2006). The results from this study show that the interaction of CRT and Zataria Multiflora supplementation is the most effective way to modulate ghrelin, leptin and adiponectin plasma levels in postmenopausal women. However, it seems, leptin and adiponectin were affected significantly by just Zataria Multiflora. Mpalaris et al. (2016) showed that there is an association between bone mineral content and those peptides, in a way that there is a positive correlation between leptin concentration and negative one among the adiponectin and ghrelin and bone mineral content of postmenopausal women. Tsaroucha et al. (2013) reported that all of these peptides singly could play a main role to mediate the pathogenesis of asthma in obese women. Therefore, according to these reports and the data from this study, it seems, Zataria Multiflora with its anti-inflammatory and metabolic effect could be a useful supplement for people who are subjected to the mentioned diseases, such as postmenopausal women. However, the interaction of CRT and Zataria Multiflora appears more efficacious.

FGF21

It is a novel factor that has been shown to possess beneficial effects on lipid metabolism and insulin sensitivity in animal studies (Fève et al., 2016). Its cooperation with adipocytes leads to stimulate insulin-independent glucose uptake by protein expression of GLUT4, and it is a key target for transcription factor PPARa, which is involved in lipid metabolism (Fève et al., 2016). Overexpression of FGF21 in transgenic mice resulted in the resistance to diet-induced obesity and metabolic perturbation (Fève et al., 2016). More importantly, it has been shown, FGF21 has a role in modulation of lipid profile (Kharitonenkov et al., 2007), which probably is due to its role in activation of extracellular signal-regulated kinase 1/2 and Akt signaling pathways (Wente et al., 2006). With regard to our results, Zataria Multiflora did not have as substantial role a ad CRT to induce a rise FGF21 plasma levels in postmenopausal women, likely this is because of the deregulation of other hormones in these women, which lead to more resistance of lipid and glucose metabolism among them (Maggio et al., 2007). However, this point requires further researches. A limitation of the current study was that the strength gain was not assessed after 4 weeks training to adjust the exercise-training protocol. This may have helped to better explain the results.

In conclusion, our findings suggest CRT is a critical intervention element that can significantly modulate circulating levels of adipokines. Interestingly, these effects were intensified when Zataria Multiflora supplementation added to CRT regimen, whereas ZM alone had little effects on measured adipokines. This implies that the beneficial effects of CRT on circulating levels of adipokines could be amplified with simultaneous intake of ZM; that is, they act synergistically. Consequently, CRT alone or its combination with ZM could be considered by researchers and health practitioners as a non-pharmacologic means to modulate and/or minimize menopause-induced obesity through alleviating the increase risk of metabolic syndrome in this population. Hence, this could lead to improved postmenopausal women's well being and health. It is also paramount to note that further studies are warranted to elucidate the potential specific mechanism(s) responsible for ZM supplementation and exercise training-induced alterations in circulating levels of adipokines.

ETHICS STATEMENT

The whole study was approved by the Ethical Committee on Human Research (ECHR) of the University of Mazandaran (Iran) according to the declaration of Helsinki.

AUTHOR CONTRIBUTIONS

All the authors contributed to the conception and design of the study and to the data collection. AS, MA, AA-D, FM, AH, SS, GB, ABA, and HZ performed the data analysis and interpretation. AS, GJ, MA, FM, SS, GB, and HZ drafted the manuscript. GJ, AS, MA, AA-D, FM, AH, SS, GB, ABA, and HZ revised, read and approved the submitted version.

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Concentric and Eccentric Endurance Exercise Reverse Hallmarks of T-Cell Senescence in Pre-diabetic Subjects

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The peripheral T-cell pool undergoes a striking age associated remodeling which is accelerated by progressive insulin resistance. Exercise training is known to delay several aspects of T-cell senescence. The purpose of the current study was to investigate the effect of 3 weeks regular concentric or eccentric endurance exercise training on the composition of the T-cell compartment in pre-diabetic subjects. Sixteen male older adults with impaired glucose tolerance were recruited and performed either concentric exercise (CE) or eccentric exercise (EE) walking 3 times a week for 3 weeks. Fasting venous blood sampling was performed before training and after the training intervention. Various T-cell subpopulations were analyzed by flow cytometry. We did not find significant time × group effects (interaction) but found several significant time effects for cell type ratios and cell subsets proportions. There was an increase of the $CD4^+/CD8^+$ (0.55 ± 0.85%; $p = 0.033$) and $CD4^+/CD3^+$ ratio (5.63 ± 8.44%; $p = 0.018$) and a decrease of the $CD8^+/CD3^+$ ratio (−0.95 ± 1.64%; $p = 0.049$) after training. We found proportional increases of $CD4^+/CCR7^+/CD45RO^+$ central memory cells (5.02 ± 7.68%; $p = 0.030$), naïve $CD8^+/CCR7^+/CD45RO^-$ (3.00 ± 6.68%; $p = 0.047$) and $CD8^+/CCR7^+/CD45RO^+$ central memory cells (3.01 ± 3.70%; $p = 0.009$), while proportions of $CD4^+/CCR7^-/CD45RO^-$ TEMRA cells (−2.17 ± 4.66%; $p = 0.012$), $CD8^+/CCR7^-/CD45RO^-$ TEMRA cells (−5.11 ± 7.02%; $p = 0.018$) and $CD16^+$ cells (−4.67 ± 6.45%; $p = 0.016$) decreased after training. 3 weeks of either CE or EE were effective in reversing hallmarks of T-cell senescence in pre-diabetic subjects. It is suggested that exercise stimulates production and mobilization of naïve T-cells, while differentiated TEMRA cells might disappear by apoptosis.

Keywords: TEMRA cells, naïve T-cells, eccentric exercise, concentric exercise, inflammation

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Abbreviations: ANCOVA, analysis of covariance; ANOVA, analysis of variance; CE, concentric exercise; CMV, cytomegalovirus; EE, eccentric exercise; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; IL-6, interleukin 6; IRP, immune risk phenotype; RPE, rate of perceived exertion; TEMRA, terminally differentiated effector memory cells; TNF-α, tumor necrosis factor-α.

INTRODUCTION

Due to increases in human life expectancy, aging is regarded as one of the biggest health issues worldwide. During aging, several functions of the immune system experience dramatic changes, which fundamentally affect health and survival. This process termed "immunosenescence" is complex and affects almost most cellular and humoral components of immunity. Clinically, these changes have noticeable consequences for the effectiveness of immune responses, the vulnerability to infection, the efficacy of vaccination, and reactivation of latent viruses in aging individuals. Together, these progressive deficits result in increased morbidity and mortality (Castle, 2000; Pawelec et al., 2010).

In particular, the T-cell pool undergoes a striking age-associated remodeling process. While peripheral cell numbers do not change, the relative composition of T-cell subpopulations is importantly affected. A major component of this change is represented by an inverted $CD4^+/CD8^+$ T-cell ratio which appears in parallel to a decline in naïve T-cell numbers and the accumulation of highly differentiated memory cells (Pawelec et al., 2010). Mostly, these cells are CD8-positive T-cells which lack the expression of the lymphoid homing receptor CCR7 and costimulatory molecules such as CD28 alongside re-expressing CD45RA, termed terminally differentiated effector memory (TEMRA) cells (Weinberger et al., 2007; Arnold et al., 2011). TEMRA cells are known to produce large amounts of pro-inflammatory cytokines suggesting that they are involved in the development of a systemic low grade inflammation during aging. The reasons for the progressive appearance of these cells are suggested to be the persistent exposure to pathogens, frequent reactivations of latent viruses, and increased levels of oxidative stress (Effros et al., 2003; Simpson et al., 2016). The concomitant increase of proinflammatory molecules during aging, particularly TNF-α (Bruunsgaard et al., 2003) and IL-6 (Cohen et al., 1997), is suggested to amplify T-cell differentiation and senescence (Di Mitri et al., 2011). Accordingly, critical age related T-cells changes are accelerated by the existence of metabolic risk factors like insulin resistance or type 2 diabetes (Ventura et al., 2017). In the end, TEMRA cells may show an impaired immune function due to a series of molecular changes and loss of co-stimulatory molecules (Goronzy et al., 2012). The general importance of the age associated changes in the T-cell repertoire is highlighted by their inclusion in the IRP. The IRP represents a cluster of immunological parameters, which was shown to be associated with a poor immune function in elderly and predictive of earlier mortality (Wikby et al., 1998).

Regular exercise has been shown to significantly improve several aspects of immune function that decline with ageing (Walsh et al., 2011). With regard to T-cells, exercise training in mice improved T-cell proliferation in response to an antigen encounter (Liu et al., 2017). In aging mice it was demonstrated that exercise restored the percentages of naïve and memory cells in the spleen toward that of young mice (Woods et al., 2003). Untrained humans exhibited higher proportions of late-stage differentiated $CD4^+$ and $CD8^+$ cells and lower proportions of naïve $CD8^+$ cells compared to trained individuals (Brown et al., 2014). Therefore, it is proposed that regular training might delay the onset of T-cell ageing or even has rejuvenating effects. While in most therapeutic interventions concentric endurance exercise is used to stimulate patients' immune system, eccentric endurance exercise has also been shown to affect T-cell immunity and metabolisms (Jensen and Richter, 2012; Peake et al., 2015). Compared to concentric endurance exercises such as uphill walking, eccentric endurance exercises such as downhill walking induce higher strain on the muscle while the metabolic cost remains relatively low (Johnson et al., 2002; Camillo et al., 2015). Thus, especially physically unfit subjects that one can find in a population of middle aged men suffering from pre-diabetes, could profit from an exercise form like downhill walking that is less demanding for the cardiovascular system than uphill walking (Drexel et al., 2008; Philippe et al., 2017).

The aim of the current study was to investigate the effects of a 3 weeks regular concentric (uphill walking) and eccentric (downhill walking) endurance exercise training in a real world setting (e.g., summer holidays in the Alps), on the composition of the T-cell compartment in pre-diabetic subjects. We hypothesize that exercise training induces an increase of naïve T-cells in parallel to a decrease of cells with a senescent phenotype. We further hypothesized that concentric and EE exhibit different effects.

MATERIALS AND METHODS

Study Participants

Data were collected in the course of the interventional study performed by Philippe et al. (2017). Therefore, the additional analysis performed for the current study are described extensively, whereas a more detailed study protocol description can be found elsewhere (Philippe et al., 2017).

For this study, the same 16 male community-dwelling older adults with IGT (age: 57.0 ± 5.2 years, BMI: 28.1 ± 2.2 $kg \cdot m^{-2}$, body mass: 86.2 ± 10.2 kg, fasting plasma glucose: 6.8 ± 1.32 $mmol \cdot l^{-1}$) were analyzed. All subjects had an IFG [defined as: IFG: fasting plasma glucose 100 $mg \cdot dl^{-1}$ (5.6 $mmol \cdot l^{-1}$) to 125 mg dl^{-1} (6.9 $mmol \cdot l^{-1}$)] and/or IGT [IGT: 2-h plasma glucose in the 75-g oral glucose tolerance test 140 mg·dl-1 (7.8 $mmol \cdot l^{-1}$) to 199 $mg \cdot dl^{-1}$ (11.0 $mmol \cdot l^{-1}$)] and did not receive any glucose metabolism relevant medication. Exclusion criteria were acute or chronic diseases, smoking, and a BMI $> 30\ kg \cdot m^{-2}$. Subjects were randomly assigned to the CE (= uphill walking, N = 8) or the EE (= downhill walking, N = 8) group. All participants gave their written informed consent to participate in the study, which was performed according to the declaration of Helsinki and approved by the ethics committee of the Medical University of Innsbruck (Protocol ID: AN5029; ClinicalTrials.gov ID: NCT01890876).

Exercise Protocol and Blood Sampling

A group of 8 participants (4 CE, 4 EE) performed the exercise protocol in spring 2013 and a group with another 8 participants in fall 2013 (4 CE, 4 EE). The training consisted of nine uphill

walking exercise sessions for the CE group and nine downhill walking sessions for the EE group, which were performed on Mondays, Wednesdays, and Fridays on 3 consecutive weeks. The starting point for the CE group was 850 m above sea level and the finish point 1360 m above sea level. Both groups were brought to their starting point by car. During each uphill walking session 510 high meters and a horizontal distance of 5 km had to be covered at a pace perceived as somewhat hard [= Borg 13], without exceeding a RPE of 15 on the 6–20 Borg scale (Borg, 1970). The EE group walked the same path as the CE group but in the opposite direction. The EE participants were asked to walk as fast as possible or at a maximum intensity corresponding to RPE 15 but without running. The total estimated energy expenditure of the 9 walking sessions was 20785 ± 4232 kJ for the CE group and 10593 ± 2211 kJ for the EE group (Philippe et al., 2017).

Fasting venous blood sampling was performed at the same time of day, before training and 1–2 days after the last walking session.

Analysis of Plasma Cytokines and Cytomegalovirus (CMV) Serostatus

The concentrations of TNF-α (TNF-α) and IL-6 in plasma were analyzed by using commercially available ELISA kits (R&D systems, Minneapolis, MN, United States). CMV serostatus was analyzed using a specific ELISA against CMV-IgG antibodies using a commercially available kit (Siemens, Germany).

Analysis of T-Cell Subpopulations

PBMCs (peripheral blood mononuclear cells) were isolated by density gradient centrifugation using Ficoll paque (VWR, Austria) and frozen (−196°C) for analysis at a later time point. Analysis of T-cell subpopulations was performed by flow cytometry (FACSCANTO II, BD, United States) using specific labeled antibodies against CD3 (APCCy7), CD4 (APC), CD8 (BV421 or PECy7), CD45RO (PE), CCR7 (FITC), CD16 (FITC), CD19 (PE), and CD25 (PE) all from BD/Europe, Biozym/Germany or eBioscience/Austria and the viability dye 7-AAD (Sigma/Germany). At first, lymphocytes were gated on a forward scatter/side scatter (FSC/SSC) dot plot following gating on living cells and CD3/CD4 or CD3/CD8. Combinations of surface markers were classified to define the respective immune cell subpopulations as stated in **Table 1** and **Figure 1**. For quality control, we followed standard procedures of the lab, which includes determination of the % bright bead robust CV for the measurements according to the manufacturer's instructions.

Statistical Analysis

Analysis was performed with the SPSS statistical software package (ver. 23.0; SPSS Inc., Chicago, IL, United States). Metric variables were visually (boxplot and skewness) and mathematically (Kolmogorov-Smirnov test) assessed for normal distribution. Means and standard deviations (SD) were calculated as descriptive statistics. A few parameters were not normally distributed in the CE or in the EE group. As there were no outliers, a sensitivity analysis was performed (repeated measures ANOVA vs. Wilcoxon test; repeated measures ANOVA vs. Mann-Whitney *U*-test) for these parameters. The sensitivity analyses showed a difference for $CD4^+/CCR7^-/CD45RO^-$ cells and $CD8^+/ CCR7^+/CD45RO^-$ cells, therefore, we opted for non-parametric testing for these variables (Wilcoxon test for effects of training over time; Mann-Whitney *U*-test for time × group effects, using the mean differences of after exercise minus

TABLE 1 | Proportional change of T-cell subpopulations before and after CE training.

					ANOVA		ANCOVA
Cell type (ratio)	**Before CE**	**After CE**	**Before EE**	**After EE**	**Main effect time *p*-value (effect size eta²)**	**Interaction time x group *p*-value (effect size eta²)**	**Group (effect size eta²)**
$CD4^+/CD8^+$	1.86 ± 0.81	2.56 ± 1.22	2.81 ± 1.08	3.19 ± 1.39	0.033* (0.306)	0.491 (0.037)	0.723 (0.012)
$CD4^+/CD3^+$	38.40 ± 13.68	47.76 ± 10.71	43.63 ± 9.92	45.00 ± 9.99	0.018* (0.362)	0.064 (0.239)	0.599 (0.026)
$CD8^+/CD3^+$	23.19 ± 9.38	22.26 ± 8.76	16.93 ± 5.77	15.94 ± 6.05	0.049* (0.266)	0.946 (<0.001)	0.658 (0.017)
Cell type (% of all lymphocytes)							
$CD4^+/CCR7^+/CD45RO^-$	43.78 ± 12.79	41.63 ± 12.02	52.56 ± 16.64	51.40 ± 11.94	0.499 (0.036)	0.836 (0.003)	0.373 (0.073)
$CD4^+/CCR7^+/CD45RO^+$	27.83 ± 8.62	32.98 ± 12.26	31.63 ± 9.29	36.50 ± 5.78	0.030* (0.313)	0.947 (<0.001)	0.844 (0.003)
$CD4^+/CCR7^-/CD45RO^+$	18.58 ± 6.36	18.55 ± 7.51	12.70 ± 8.24	10.20 ± 7.00	0.367 (0.630)	0.376 (0.061)	0.740 (0.010)
$CD4^+/CCR7^-/CD45RO^-$	9.84 ± 12.36	6.83 ± 7.02	3.13 ± 1.81	1.91 ± 0.84	0.012* (0.186)	0.908 (0.040)	0.740 (0.010)
$CD25^+/CD4^+$	29.91 ± 8.05	33.34 ± 8.62	39.36 ± 8.80	37.56 ± 11.21	0.740 (0.009)	0.295 (0.084)	0.654 (0.019)
$CD19^+$ lymphocytes	9.44 ± 4.72	16.34 ± 22.76	8.24 ± 5.87	6.86 ± 4.72	0.497 (0.036)	0.313 (0.078)	0.640 (0.021)
$CD16^+$ (lymphocytes)	24.10 ± 14.86	17.60 ± 8.24	16.63 ± 12.38	14.06 ± 9.76	0.016* (0.369)	0.253 (0.099)	0.216 (0.136)
$CD8^+/ CCR7^+/CD45RO^-$	19.69 ± 15.19	23.16 ± 16.66	28.69 ± 19.11	31.14 ± 18.38	0.047* (0.175)	0.817 (0.006)	0.482 (0.046)
$CD8^+/CCR7^+/CD45RO^+$	5.61 ± 4.00	9.31 ± 5.83	8.86 ± 3.80	11.09 ± 7.04	0.009* (0.417)	0.463 (0.042)	0.315 (0.092)
$CD8^+/CCR7^-/CD45RO^+$	30.15 ± 15.75	28.91 ± 15.86	26.90 ± 9.52	26.41 ± 8.41	0.654 (0.016)	0.844 (0.003)	0.156 (0.174)
$CD8^+/CCR7^-/CD45RO^-$	44.58 ± 17.86	38.65 ± 14.57	35.53 ± 12.78	31.36 ± 10.10	0.018* (0.359)	0.647 (0.017)	0.726 (0.012)
$CD25^+/CD8^+$	5.50 ± 1.43	6.86 ± 3.39	10.94 ± 6.22	10.77 ± 3.96	0.383 (0.059)	0.265 (0.094)	0.009* (0.475)

*$p < 0.05$.

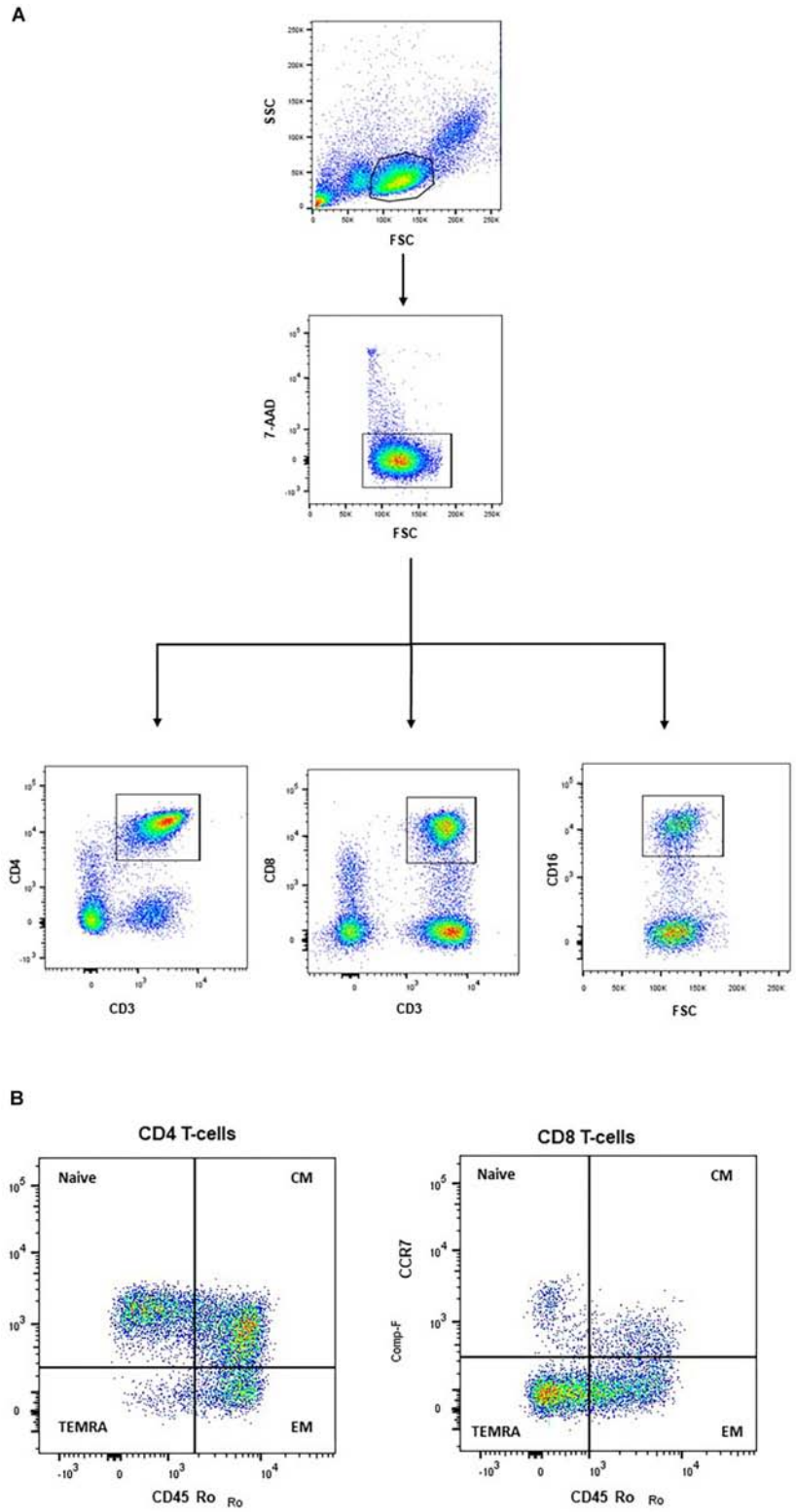

FIGURE 1 | Frozen PBMCs were defrosted and stained with fluorochrome-conjugated antibodies. Gating strategy: Lymphocytes were gated in a FSC/SSC-dot plot and dead cells excluded using 7-AAD. From the living cell population, CD4 T-cells, CD8 T-cells and $CD16^{+}$ cells were gated according to their specific surface markers **(A)**. T-cell subpopulations, within CD4 and CD8 T-cells, respectively, were defined by staining CD45RO and CCR7. NAIVE (CD45RO-/CCR7+): naive T-cells; CM (CD45RO+/CCR7+): central memory; EM (CD45RO+/CCR7-): effector memory; TEMRA (CD45RO-/CCR7-): effector memory + RA **(B)**.

before exercise values). For all other variables, the sensitivity analyses showed no marked differences between parametric and non-parametric testing, thus we opted to stick with the more robust parametric tests (Field, 2011). The effect of training over time (before and after), as well as the time x group effect (interaction) for cytokines, T-cell subpopulation, $CD19^+$ B lymphocytes and $CD16^+$ cells were assessed by repeated measures ANOVA, with group as the between-subjects factor. In addition, effect sizes (partial eta squared, eta^2) were also calculated. As several cell parameters were different at baseline when comparing CE with EE, we performed an ANCOVA for all parameters with the pretest values as a covariate, to test, whether there was a difference in the post-test means by group after adjusting for the pretest observation. Relations between CMV serostatus and T-cell subpopulations before and after exercise as well as relations between changes of plasma cytokine levels and changes of T-cell subpopulation, $CD19^+$ B lymphocytes and $CD16^+$ cells were calculated with Pearson product-moment correlation coefficient. To assess whether there was a difference in the frequency of CMV-positive and CMV-negative participants between CE and EE, we performed a chi-squared test. Statistical significance level was set at $p < 0.05$.

RESULTS

We were not able to detect time x group (interaction) effects for any of the investigated parameters, i.e., the analysis of plasma cytokines (**Table 2**), and the analysis of T-cell subpopulations.However, time effects on cell type ratios and cell subsets proportions could be observed in both groups following eccentric and concentric training:

The ratios of $CD4^+/CD8^+$ ($F_{(1,13)} = 5.725$; $p = 0.033$) and $CD4^+/CD3^+$ ($F_{(1,13)} = 7.725$; $p = 0.018$) significantly increased after training, while the $CD8^+/CD3^+$ ratio ($F_{(1,13)} = 6.815$; $p = 0.049$) significantly decreased after training (**Table 2**).

We found a significant increase of the relative proportion of $CD4^+/CCR7^+/CD45RO^+$ central memory cells ($F_{(1,13)} = 5.912$; $p = 0.030$) and a significant decrease of $CD4^+/CCR7^-/CD45RO^-$ TEMRA cells ($p = 0.012$) and the relative proportion of $CD16^+$ cells ($F_{(1,13)} = 7.617$; $p = 0.016$) after training (**Table 1**).

Training significantly increased the relative proportion of $CD8^+/CCR7^+/CD45RO^+$ central memory cells ($F_{(1,13)} = 9.282$; $p = 0.009$) and $CD8^+/ CCR7^+/CD45RO^-$ naïve cells ($p = 0.047$), and significantly decreased the relative proportion of $CD8^+/CCR7^-/CD45RO^-$ TEMRA cells ($F_{(1,13)} = 7.286$; $p = 0.018$; **Table 1**).

ANCOVA showed no significant group effect for the above mentioned parameter thus, we may assume that baseline differences between groups may not account for the observed time effects.

At baseline, 9 participants were CMV-positive and 7 participants were CMV-negative. Chi-squared test showed no difference in CMV serostatus (positive or negative) between CE and EE ($p = 0.131$). CMV serostatus neither correlated with any T-cell subpopulation before exercise nor with exercise induced changes of T-cell subpopulations. Changes of TNF-α plasma levels significantly positively correlated with changes of the $CD16^+$ cells proportion ($R = 0.533$; $p = 0.041$).

DISCUSSION

The current study demonstrates that 3 weeks of concentric or eccentric endurance training were able to increase both, the $CD4^+/CD8^+$ ratio as well as the $CD4^+/CD3^+$ ratio in pre-diabetic subjects, indicating a relative increase in $CD4^+$ T helper cells. The proportional increase of naïve and of central memory $CD8^+$ cells after CE and EE indicates a shift to lower differentiated cell types. In parallel, the proportion of $CD8^+$ TEMRA cells decreased indicating a relative reduction of highly differentiated T-cells. Surprisingly, despite CE and EE being different training modalities, inducing different stress on the contractile filaments of muscle and the muscle metabolism (Camillo et al., 2015; Philippe et al., 2017), we found no interaction effects regarding changes of cytokine plasma levels, cell ratios or $CD4^+$ and $CD8^+$ T-cell subset proportions.

A decreased $CD4^+/CD8^+$ ratio is an accepted hallmark of immunological ageing. In a series of longitudinal studies of octogenarians in exceptional health, and nonagenarians, this parameter was identified as part of the "Immune Risk Profile" (IRP) (Pawelec et al., 2010). Accordingly, it represents an important characteristic which predicts a greater all-cause mortality (Wikby et al., 1998). Chronic diseases which are accompanied by a systemic low grade inflammation, have been shown to amplify the decrease of the $CD4^+/CD8^+$ ratio, resulting in a compromised host protection (Richard et al., 2017). The current data demonstrate that 3 weeks of regular CE or EE were followed by an increase of the $CD4^+/CD8^+$ ratio. The concomitant increase of the $CD4^+/CD3^+$ ratio indicates that these changes are due to an increase of $CD4^+$ T helper cells. The mechanisms for these changes are speculative. On the one hand, each bout of acute exercise mobilizes hematopoietic stem cells into blood which might travel to the thymus or extrathymic places for differentiation into naïve $CD4^+$ cells (Krüger et al., 2015). On the other hand, exercise might mobilize more differentiated T-cells from the marginal pool and the spleen into the circulation (Krüger et al., 2008). Both mechanisms might contribute to altered $CD4^+$ cell numbers. Regarding the slight decrease of the CD8/CD3 ratio, it is suggested that the relative number of $CD8^+$ T-cells is lower after training. This assumption is supported by previous data from longitudinal training studies which have also reported declines in total $CD8^+$ T-cells after 12 weeks and 6 months of endurance training (Baj et al., 1994; Gleeson et al., 2000).

Besides a general increase of $CD4^+$ T helper cells, we also found changes in $CD4^+$ T-cell subsets. While the proportion of $CD4^+$ central memory cells increased, there was a decrease of $CD4^+$ cells with TEMRA cell characteristics after training, without differences between CE and EE. These alterations represent a shift from more differentiated to less differentiated cells in the $CD4^+$ T helper cell compartment. However, actually the concept of the $CD4^+$ equivalent to TEMRA cells is intensively

TABLE 2 | Cytokine levels before and after CE and EE training.

Cytokine levels	Before CE	After CE	Before EE	After EE	ANOVA	ANCOVA
					ANOVA Main effect time *p*-value (effect size eta^2)	ANCOVA Group (effect size eta^2)
TNF-α (pg·ml^{-1})	1.99 ± 1.16	1.77 ± 0.60	1.43 ± 0.37	1.59 ± 0.68	0.862 (0.002)	0.760 (0.007)
IL-6 (pg·ml^{-1})	2.06 ± 0.90	1.83 ± 1.77	1.84 ± 1.24	2.84 ± 2.72	0.490 (0.035)	0.317 (0.077)

*$p < 0.05$.

discussed and not clearly defined. Therefore, it is difficult to conclude any immunological impact of changes in this subpopulation (Larbi and Fulop, 2014).

With regard to CD8$^+$ cells, changes in T-cell subsets were found after training, without differences between CE and EE. For naïve and central memory CD8$^+$ T-cells, a proportional increase was found. Both cell types are known to be effective in response to invading antigens by having a high capacity to expand and proliferate (Sallusto et al., 2004). With regard to immunosenescence these changes seem to be important because decreased numbers of naïve CD8 T-cells represent an important biomarker of an ageing immune system (Appay and Sauce, 2014). The age-related reduction of naïve CD8 cells is suggested to be due to reduced T-cell maturation as a result of thymic involution, but also to reduced generation of lymphoid progenitors and to a higher susceptibility to death receptor-mediated apoptosis, triggered by peripheral inflammatory cytokines like TNF-α (Messaoudi et al., 2004; Emmons et al., 2017). Exercise is suggested to effectively affect all these processes. Accordingly, each acute bout of exercise mobilizes hematopoietic progenitor cells into blood (Krüger et al., 2015). It was further shown that T-cells of trained subjects exhibit an increased resistance to apoptosis (Krüger and Mooren, 2014). Finally, exercise training has anti-inflammatory properties resulting in reduced systemic TNF-α levels (Pedersen and Bruunsgaard, 2003). However, since we did not find a significant reduction of either, TNF-α or IL-6 after training, this mechanism is unlikely.

After CE and EE, a decrease of CD4$^+$/CCR7$^-$/CD45RO$^-$ and CD8$^+$/CCR7$^-$/CD45RO$^-$ cells was found, implicating a proportional reduction of TEMRA cells. An accumulation of TEMRA cells, specifically CD8$^+$ T-cells, is an important feature of immune aging since these cells produce large amounts of pro-inflammatory cytokines after activation (Zhang et al., 2001). In addition, CD8 TEMRA cells have only a reduced capacity to proliferate, suggesting that an increase of these cells reduces an organism's capacity to defend itself against invading pathogens. The age-related increase of TEMRA cells is even more pronounced in the T-cell pool of CMV-seropositive elderly compared to CMV-seronegative elderly persons (Simpson et al., 2016). Therefore, the CMV serostatus was analyzed, but could be excluded as a significant confounder in the present study. The reduced proportion of CD8$^+$TEMRA cells after exercise is speculated to be the result of at least two different processes. On the one hand, the relative increase of other CD8 positive T-cell subpopulations might reduce the relative proportion of TEMRA cells. On the other hand, it was shown that acute bouts of exercise induces apoptosis in T-cells which was more frequently observed in more differentiated cells (Krüger et al., 2016). Integrating the exercise-induced increase of naïve T-cells and decrease of TEMRA T-cells in a holistic model, there might exist a negative feedback loop between cell death and mobilization of progenitor cells. Such a connection has been demonstrated in mice, where application of apoptotic bodies evoked the mobilization of hematopoietic progenitors. Accordingly, the loss of senescent cells by apoptosis might create space for the expansion of naïve T-cells (Simpson, 2011; Mooren and Krüger, 2015). However, since we did not analyze either apoptosis or progenitor cell mobilization, these mechanisms have to be addressed in future projects.

CD16$^+$ represent a subgroup of lymphocytes which include NK cells, gamma-delta T-cells and subsets of CD8$^+$ T-cells. The correlation analyses showed a significant positive correlation between changes of TNF-α and changes of CD16$^+$ cells. Thus, the participants with the highest reductions of CD16$^+$ cells exhibited the most important decreases of TNF-α plasma levels. While a direct link between these processes is speculative, both processes might reflect the anti-inflammatory effect of exercise training. Consequently, especially persons suffering from pre-diabetes may benefit from these alterations as TNF-α mediated low-grade inflammation is co-responsible for the development of insulin resistance (Pedersen, 2017).

Despite the lower estimated energy expenditure of the EE group compared to the CE group all observed effects were not different between CE and EE. It has previously been shown that despite the lower energy cost, EE had equal or even superior positive effects on glucose and lipid metabolism and inflammation (Drexel et al., 2008; Paschalis et al., 2010). Our results indicate that these findings may also be true regarding positive alterations of immunosenescence. Thus, uphill and downhill walking may equally be recommended for older persons and especially for subjects suffering from pre-diabetes.

Limitations

The relatively small sample size may be considered as a limitation. Furthermore, the present investigation misses a control group. Yet, as the main aim of this study was to compare the effects of CE and EE on the composition of the T-cell compartment this limitation might be considered minor. Furthermore, we neither measured CD56$^+$ surface marker in order to specifically characterize NK cells and neither monitored diet and caloric intake nor physical activity patterns during the study phase. Although we explicitly asked the study participants not to change

their eating, drinking or physical activity habits during the study period, we cannot entirely rule out that additional life style changes might have influenced our results. In contrast to studies displaying indoor or laboratory training regimens, outdoor training cannot be comparably standardized. For example, participants had to encounter changing weather conditions, also due to different seasons. These uncontrollable external factors may have influenced the results.

CONCLUSION

In conclusion, the current data implicate that 3 weeks of either concentric or eccentric endurance training increases the proportion of $CD4^+$ T-cells and of naïve $CD8^+$ T-cells and central memory $CD4^+$ and $CD8^+$ T-cells, and reduces the proportion of $CD4^+$ and $CD8^+$ TEMRA cells in pre-diabetic subjects. These changes might be favorable for patients since they represent an opposing trend against immunosenescence and might stimulate host defense against invading pathogens.

ETHICS STATEMENT

All participants gave their written informed consent to participate in the study, which was performed according to the declaration of Helsinki and approved by the ethics committee of the Medical University of Innsbruck (Protocol ID: AN5029; ClinicalTrials.gov ID: NCT01890876).

AUTHOR CONTRIBUTIONS

MP, MB, and BG-L conceived and designed the research. MP, HG, BW, MB, and MK performed the experiments. MP, HG, MK, BW, and KK analyzed the data. KK and MP drafted the manuscript. MP, HG, MK, KA, JF, MB, BG-L, and KK edited and revised the manuscript. MP, HG, MK, BW, KA, JF, MB, BG-L, and KK approved final version of the manuscript.

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frontiers in Physiology

MINI REVIEW
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Resistance Exercise Training as a Primary Countermeasure to Age-Related Chronic Disease

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Age is a primary risk factor for a number of chronic diseases including mobility disability, cardiovascular disease (CVD), type 2 diabetes (T2D), and cancer. Most physical activity guidelines emphasize the performance of 150 min of moderate-to-vigorous or 75 min of vigorous aerobic exercise training (AET) weekly for reduction of chronic disease risk. Nonetheless, there is an emerging body of evidence showing that resistance exercise training (RET) appears to be as effective as AET in reducing risk of several chronic diseases. It may also be that RET is more effective than AET in some regards; the converse is likely also true. We posit that the perceived divergent exercise mode-dependent health benefits of AET and RET are likely small in most cases. In this short review, our aim is to examine evidence of associations between the performance of RET and chronic health disease risk (mobility disability, T2D, CVD, cancer). We also postulate on how RET may be influencing chronic disease risk and how it is a critical component for healthy aging. Accumulating evidence points to RET as a potent and robust preventive strategy against a number of chronic diseases traditionally associated with the performance of AET, but evidence favors RET as a potent countermeasure against declines in mobility. On the basis of this review we propose that the promotion of RET should assume a more prominent position in exercise guidelines particularly for older persons.

Keywords: aging, sarcopenia, physical mobility, resistance training, chronic disease risk, cardiovascular disease, type 2 diabetes, cancer

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INTRODUCTION

Cardiovascular disease (CVD), cancer, and type 2 diabetes (T2D) are leading causes of morbidity and mortality in older adults aged 65 years and older (Roth et al., 2015; Tanday, 2016). Aging is also the single biggest predictor for mobility impairments, which can exacerbate the risk for all of the aforementioned chronic diseases (Newman et al., 2006). Pharmacological agents are frequently prescribed to treat or delay the progression of major chronic diseases in mobility-impaired older individuals; however, most if not all of these therapies have some degree of off-target effects that may be undesirable or reduce compliance with prescribed dosing. Global population aging has resulted in a concomitant increase of people living with age-related chronic disease and also with impaired physical mobility. Low cost, widely implementable multi-condition pharmaceutical interventions that have a low side-effect profile and mitigate risk for all common chronic diseases while alleviating the risk of mobility decline do not presently exist. However, routine exercise can variably mitigate the age-related reduction in physical mobility and reduce chronic disease risk to an appreciable extent.

The progressive decline of skeletal muscle mass and strength with aging is collectively referred to as sarcopenia, and is prognostic for mobility disability (Visser et al., 2002, 2005) and chronic disease risk (Pedersen and Saltin, 2015). Regular physical activity (defined here as any bodily

movement produced by the contraction of skeletal muscle that increases energy expenditure; Caspersen et al., 1985) and exercise (physical activity that is planned, structured, and repetitive; Caspersen et al., 1985) are cornerstones in the primary prevention of chronic diseases (Pedersen and Saltin, 2015) and also for mitigating risk of mobility disability in older persons (Pahor et al., 2014; Villareal et al., 2017).

Resistance exercise (RE) and aerobic exercise (AE) are modalities of exercise that are traditionally conceptualized as existing on opposite ends of an exercise continuum in terms of the phenotypes they lead to. A common misconception is that RE training (RET) and AE training (AET) also result in separate health benefits, but we propose this is an artifact of the greater volume of data that currently exists for AET as opposed to RET. Currently, most physical activity guidelines advise, as their primary message, that older adults should perform at least 150 min of moderate-to-vigorous or 75 min of vigorous AET weekly for the reduction of chronic disease risk and maintenance of functional abilities (American College of Sports Medicine, 2009; Canadian Society for Exercise Physiology, 2011; American Heart Association, 2018; Piercy et al., 2018). However, there is an emerging body of evidence to suggest that RET can be as effective as AET in reducing chronic disease risk and is particularly potent for maintaining mobility in older adults (Tanasescu et al., 2002; de Vries et al., 2012; Grontved et al., 2012; Stamatakis et al., 2018).

The aim of this review is to provide an up-to-date evidence-based narrative review of the efficacy of RET in combating chronic health disease (mobility disability, T2D, CVD, and cancer) risk in older adults. To achieve this aim, we summarize data derived predominantly from humans, but will draw upon important findings from preclinical disease models to substantiate our arguments and provide additional mechanistic insight not available in human observational trials.

RESISTANCE EXERCISE TRAINING AND PHYSICAL MOBILITY

Mounting evidence from systematic reviews (Theou et al., 2011), meta-analyses (de Vries et al., 2012; Gine-Garriga et al., 2014; de Labra et al., 2015), and umbrella reviews (Jadczak et al., 2018) convincingly show that exercise interventions combining RET and AET are the most effective for combating age-related declines in physical mobility. Villareal et al. (2017) demonstrated that obese older adults with mobility limitations who performed combined (AET and RET) training improved objective and subjective measures of functional ability more than individuals randomized to either RET or AET alone. However, as is often the case in these clinical trials, the combined RET plus AET group performed a larger volume of exercise than the groups performing either modality alone, which likely confounded the results.

A recent umbrella review demonstrated that RET in pre-frail and frail older adults could significantly enhance muscular strength, gait speed, and physical performance (Jadczak et al., 2018). Pooled data from 33 randomized controlled trials showed that performing RET resulted in a statistically significant improvement in physical function (Liu and Latham, 2009). de Vries et al. (2012) have argued that RET is of greater importance in an exercise program than AET for improving physical mobility in community-dwelling, mobility-impaired older adults. On the contrary, a recent meta-analysis conducted by Hortobagyi et al. (2015) found similar improvements in gait speed in healthy older adults performing either AET or RET. The heterogeneity in experimental design across studies (i.e., participant characteristics, training variables [frequency, intensity, time]), and methods used to assess mobility, can make it difficult to conclude which exercise modality is most efficacious in combatting mobility declines in older adults. Cognizant of this limitation, future randomized controlled trials are warranted to investigate which exercise mode is most effective in improving physical function in older adults. Nevertheless, when looked at collectively, the evidence suggests that RET can play a fundamental role in improving and/or maintaining functional mobility that is at least on par with those imbued by AET, in older adults.

The underlying mechanisms by which RET attenuates the decline in physical function of older adults is likely multifaceted. However, low muscle mass and strength are associated with poor physical function (Visser et al., 2002), and predictive of future mobility impairment in older adults (Visser et al., 2005). A recent cross-sectional analysis determined that community-dwelling older adults with low muscle mass and combined low muscle mass and function had a 1.6- and 12.2-increased odds of being physically dependent, respectively (Dos Santos et al., 2017). RET is a potent stimulus for skeletal muscle hypertrophy and augmenting strength in older adults. Indeed, a meta-analysis containing 49 randomized controlled trials concluded that after an average of 20.5 weeks of RET, older adults gained 1.1 kg of lean body mass (Peterson et al., 2011). Moreover, RET (either alone or as part of a combined training program) enhanced strength gains in frail older adults more than combined exercise programs without RET. Whole-body progressive RET (2 sets of 65–85% of 1 repetition maximum [1RM]) 3 times a week for 6 months attenuated losses in bone mineral density, lean mass, and muscular strength in obese frail participants to a greater extent than combined training or AET (jogging/running for 60 min at 65–85% of heart rate peak [HR_{peak}]; Villareal et al., 2017). In contrast, AET alone is ineffective at inducing comparable increases in skeletal muscle mass and strength (Grgic et al., 2018). In addition, RET can improve neurological (i.e., increased central motor drive, elevated motoneuron excitability; Aagaard et al., 2002), psychological (i.e., self-efficacy; Kekalainen et al., 2018), and/or cardiovascular function (i.e., maximal stroke volume; Roberson et al., 2018) – all of which have been hypothesized to contribute to skeletal muscle performance in older adults (Tieland et al., 2018). Thus, it is not surprising that RET exerts beneficial effects on physical function in older adults – regardless of whether muscle hypertrophy is observed – through factors extrinsic to skeletal muscle. Further work is needed to identify the dominant mechanism by which RET can combat mobility impairments.

Although high-intensity RET (≥70% of 1RM) is generally more effective than low-to-moderate intensity RET (30–69% of 1RM)

in combating mobility decrements (de Vries et al., 2012), the heterogeneity between studies makes it is difficult to conclude with a high degree of certainty the optimal RET intensity. It should be noted that RET where one's own body weight is used for resistance and in which activities of daily living are simulated (i.e., body-weight squat) can improve indices of physical function in older adults to a similar extent as conventional RET (requiring external loads; Lustosa et al., 2011). Notwithstanding, multicomponent exercise programs (consisting of RET, AET, and balance training in combination) appear to be the best strategy for attenuating declines in physical mobility (Theou et al., 2011; de Vries et al., 2012; Cadore et al., 2013; Gine-Garriga et al., 2014; de Labra et al., 2015; Jadczak et al., 2018).

RESISTANCE EXERCISE TRAINING AND TYPE 2 DIABETES

A hallmark of aging is the progressive deterioration of whole-body insulin sensitivity and consequent impairment of glycemic control (Jackson et al., 1982) that predispose older adults to T2D. T2D is the nexus of insulin resistance and impaired β- cell function (Weyer et al., 2001), and is one of the most prevalent metabolic diseases afflicting older adults (Statistics Canada, 2016). In older adults, the insulin-mediated suppression of hepatic glucose output is delayed and peripheral glucose uptake into skeletal muscle is impaired (Jackson et al., 1982). Inevitably, the inability of the aging pancreas to produce and secrete enough insulin to buffer the resistance in peripheral and hepatic tissues leads to T2D.

Given that ~80% of glucose is deposited in skeletal muscle during postprandial periods (Thiebaud et al., 1982), the loss of muscle mass and that of muscle metabolic quality with advancing age are thought to be primary drivers of insulin resistance and T2D development in older adults (DeFronzo and Tripathy, 2009). Epidemiological data (Srikanthan and Karlamangla, 2011) demonstrate an inverse relationship between lean body mass and insulin resistance, an effect that appears independent of, but exacerbated by, obesity in older adults (Srikanthan et al., 2010). Moreover, declining muscle strength and progressive mobility impairment with age likely cause a reduction in daily physical activity, which alone is sufficient to induce metabolic dysfunction (McGlory et al., 2018).

Recent work from our laboratory demonstrated that a reduction of habitual daily stepping for a period of 2 weeks (<1,000 steps/day) in prediabetic older adults results in significant impairments in glycemic control and an insulin-resistant state in response to a 75-g oral glucose challenge (McGlory et al., 2018). Importantly, participants failed to recover baseline insulin sensitivity upon returning to habitual activity for 2 weeks. Recently, Reidy et al. (2018) confirmed the induction of insulin resistance following step-reduction using a hyperinsulinemic-euglycemic clamp, the gold-standard method to assess insulin sensitivity. In contrast to our findings, however, Reidy et al. (2018) demonstrated that older adults fully recovered baseline insulin sensitivity following a return to habitual stepping. These data together suggest that reductions in physical activity levels in older adults contribute substantially to the development of insulin resistance that precedes diabetes development.

Lifestyle interventions are arguably the most effective therapeutic strategies in terms of preventing and managing diabetes. Indeed, the Diabetes Prevention Program (DPP) demonstrated that lifestyle modifications (i.e., diet and exercise) were associated with a greater reduction (58 vs. 31%; Knowler et al., 2002) in the incidence of T2D compared to metformin – the current frontline therapy for T2D (American Diabetes Association, 2014). Importantly, however, the DPP focused on AET with little consideration of the beneficial effects of RET on glycemic control. Interesting data from Davy et al. (2017) demonstrated that after only 3 months (2×/week) of progressive, supervised, whole-body RET (1 set at 70–80% 1RM), ~34% of overweight/obese prediabetic older adults achieved normal glucose tolerance. These findings are not isolated and, when considered collectively (Zachwieja et al., 1996; Iglay et al., 2007), support the effectiveness of RET to improve glycemic control in elderly adults. These improvements would be expected, and have been reported to translate into reduced T2D incidence in the elderly (Grontved et al., 2012). Indeed, an analysis of ~32,000 men between the ages of 40–75 years from the Health Professionals' Study demonstrated that men engaging in at least 150 min/week of RET had a 34% lower risk of developing diabetes over an 18-year period (Grontved et al., 2012). Model-derived estimates predict that a risk reduction of this magnitude (~30%) would save ~$1.5 billion in healthcare expenditure (Bilandzic and Rosella, 2017).

People with diagnosed type 1 and type 2 diabetes can also benefit from the inclusion of RET for the management of glycemia as an adjunct therapy to antidiabetic pharmaceutical agents (American Diabetes Association, 2018). In one study, an acute bout of either AE (running at 60% maximal oxygen uptake [VO_{2max}]) or whole-body RE (3 sets at 70% 1RM) resulted in significant reductions of plasma glucose levels in physically active type 1 diabetics (Yardley et al., 2013). Although the decrement was greater during AE, interstitial glucose monitoring post-exercise demonstrated that only the participants performing RE maintained lower plasma glucose levels over the ensuing 24 h. In a recent meta-analysis including 360 older patients with T2D, RET for at least 8 weeks was also associated with clinically relevant improvements in glycated hemoglobin (HbA1c) and muscle strength (Lee et al., 2017). The RET-induced improvement in HbA1c was also observed in 7/8 studies systematically analyzed by Gordon et al. (2009). Future randomized controlled trials are now needed to examine the salient mechanisms driving the rejuvenation of insulin sensitivity in response to RET, which are briefly considered below.

Muscle contraction *per se* improves glucose homeostasis through insulin-dependent and independent signaling pathways (Holloszy, 2005). Theoretically, growth or atrophy of skeletal muscle is expected to perturb glucose handling through expansion or contraction, respectively, of the predominant glucose disposal site; however, RET can improve insulin sensitivity independently of changes in lean body mass (Holten et al., 2004), indicating that intrinsic insulin signaling is improved. After binding to its membrane receptor, insulin initiates a signaling cascade that converges on the phosphorylation of AS160, permitting the translocation and

docking of GLUT4 transporters onto the sarcolemma and enhancing glucose uptake. Insulin-mediated phosphorylation of AS160 is impaired in older adults resulting in reduced GLUT4 delivery to the sarcolemma and decreased muscle uptake (Consitt et al., 2013). Once inside the cell, a majority of glucose is directed toward glycogen synthesis in normoglycemic adults *via* glycogen synthase activation. This process is impaired, and is thought to be a primary driver of insulin resistance, in T2D (Shulman et al., 1990; Pedersen et al., 2015). Glycogen synthase content is also reduced in aged skeletal muscle (Pastoris et al., 2000) and, together with reduced GLUT4 translocation, likely contributes to the marked reduction in peripheral glucose disposal in insulin-resistant older adults (Jackson et al., 1988). Fortunately, these age-related impairments are partially reversible with RET. For instance, sedentary older men participating in 8 weeks of combined RET and AET (training variables not published) demonstrated increased skeletal muscle hexokinase II, Akt2, glycogen synthase, and GLUT4 protein content (Bienso et al., 2015). These changes were associated with a significant decrease of insulin area under the curve during an oral glucose tolerance test (OGTT), in the absence of change in glucose area under the curve, indicating an improvement in whole-body insulin sensitivity (Bienso et al., 2015). Finally, older adults participating in RET (3 sets at 60–85% 1RM) for 24 weeks exhibited large increases (~57%) in mitochondrial oxidative capacity (Jubrias et al., 2001), which is likely linked to the training-induced improvements in insulin sensitivity.

We propose that there is good rationale and data in support of a role for RET in the prevention and treatment of insulin resistance in older adults. However, it currently remains unclear which RET training variable is most closely related with the RET-induced improvements in glycemic control in individuals with T2D. Evidence from a systematic review (Gordon et al., 2009) suggests that exercise intensity is the key variable and that performing high-intensity RET (≥70% 1RM) results in the greatest improvement in glycemic control. However, the majority of trials included in this study did not control for the total volume of exercise being performed. Indeed, a recent study in individuals with T2D demonstrated that, when matched for exercise volume, there was no significant difference in glycemic control with high- or low-intensity RET (75 vs. 50% of 1RM, respectively) (Yang et al., 2017). Further work is needed to confirm these results; nonetheless, this work provides rationale that older adults with T2D (or at high risk for developing T2D) should simply concentrate on performing RET without having to worry about the exercise intensity. The resolution of hyperglycemia and hyperinsulinemia in metabolically compromised older adults through exercise not only prevents the pathogenesis of T2D, but also the associated microvascular complications that, if unabated, are precursors to a number of comorbidities in persons with T2D.

RESISTANCE EXERCISE TRAINING AND CARDIOVASCULAR DISEASE

AET reduces the risk of CVD and mortality (Yusuf et al., 2004; O'Donnell et al., 2016), and as a result has been the focus of lifestyle interventions targeting these ailments. This observation comes as no surprise given that improved cardiorespiratory fitness – a hallmark adaptation in response to AET – is inversely associated with CVD risk and mortality (Nauman et al., 2017). In addition to cardiorespiratory fitness, muscle mass and strength are also independently associated with risk for CVD and mortality (Ruiz et al., 2008; Srikanthan et al., 2016; Kim et al., 2017), and yet RET is usually emphasized far less as an exercise modality that reduces CVD risk.

A follow-up from the Health Professional's study demonstrated that RET for at least 30 min per week resulted in a similar risk reduction compared to 2.5 h of brisk walking in fatal and nonfatal myocardial infarction (Tanasescu et al., 2002). Similarly, a recent analysis of the Women's Health study showed that women engaging in 60–120 min of RET per week had a similar 22% reduced risk of incident CVD as women engaging in 60–120 min of AET per week (Shiroma et al., 2017). Smutok et al. (1993) randomized older men at risk for developing CVD to either whole-body, progressive RET (2 sets at 60–70% 1RM) or treadmill walking/jogging (75–85% heart rate reserve) for 20 weeks and found that RET reduced risk factors associated with CVD to a similar degree as walking/jogging on the treadmill. Clearly, the aforementioned evidence suggests that RET is associated with reductions in CVD risk and mortality that are similar in magnitude as those provoked by AET.

From a mechanistic perspective, RET results in favorable improvements in a constellation of risk factors associated with CVD to the same degree as AET (i.e., blood pressure, blood lipids, insulin sensitivity, and vascular function; Yang et al., 2014). Graded increases in systolic blood pressure (SBP) and diastolic blood pressure (DBP) remain two of the most significant modifiable risk factors for CVD (Lopez et al., 2006). Meta-analyses demonstrate that RET induces reductions in SBP and DBP that are of similar or greater magnitude to AET in healthy adults (Cornelissen and Smart, 2013; MacDonald et al., 2016). Notably, the magnitude of RET-induced reductions in SBP (5–6 mmHg) and DBP (3–4 mmHg) are associated with an 18% reduction of major cardiovascular events (Blood Pressure Lowering Treatment Trialists Collaboration, 2014). The beneficial effects of RET on SBP and DBP extend to individuals with hypertension (MacDonald et al., 2016). In fact, compared to individuals with normal blood pressure, individuals with hypertension yield the largest reductions in blood pressure following RET (MacDonald et al., 2016). Considering that reductions in SBP and DBP serve as a cornerstone of CVD prevention in individuals with hypertension (Joseph et al., 2017), RET may serve as an adjunct or even alternative treatment to commonly prescribed antihypertensive medications. Future randomized controlled trials are warranted to compare RET-induced BP reductions to antihypertensive medications in individuals with hypertension.

The above-mentioned benefits of RET on cardiovascular health extend to individuals with T2D (Yang et al., 2014). Considering that compared to nondiabetic individuals, persons with T2D have a two- and fourfold risk of developing CVD, these findings are particularly important (Emerging Risk Factors Collaboration, 2010). Age-specific mortality rates of CVD fell by ~15% between 2005 and 2015 (Roth et al., 2015); however, as a consequence of the

rising prevalence of older adults living with T2D, estimates suggest an increasing proportion of cardiovascular mortality may be attributable to this metabolic condition. Although the beneficial effects of RET on cardiovascular health are clear, RET is not typically endorsed as a mode of exercise for reducing CVD risk (American Heart Association, 2018).

Clinical prescription of RET is rare largely due to the perception that AET is safer and likely easier to implement in patients with CVD. It has been suggested that high-pressure loads induced on the heart by RET can lead to a mild form of cardiac hypertrophy, which can lead to higher mortality risk (Kamada et al., 2017). However, excessive elevation of blood pressure is seen only with high-intensity RET (≥70% of 1RM) (MacDougall et al., 1985), and is generally not a concern for lighter-to-moderate intensity RET (30–69% of 1RM). Williams et al. (2007) argue that most RET studies evaluating safety have selected low-risk individuals, and that the studies do not provide reliable estimates of event rates on a population basis. However, this argument has limited supporting evidence. For example, Hollings et al. (2017) pooled together data from 5 studies evaluating adverse events during low-to-moderate intensity RET (30–69% of 1RM) in older adults with CVD, and found that RET was actually associated with a *lower* rate of adverse cardiovascular complications than AET. Furthermore, a meta-analysis in older adults at risk for developing CVD demonstrated that arterial stiffness (a correlate of cardiovascular mortality; Laurent et al., 2001) does not increase or worsen following RET (Evans et al., 2018). In fact, an acute bout of RE appears to be more protective from ischemic changes than a bout of AE, and results in a lower heart rate response and higher diastolic perfusion pressure (Featherstone and Holly, 1993). These physiological changes result in a more favorable supply of oxygen to the myocardium during RE. Thus, the misconception that RET is less safe than AET in physically or metabolically vulnerable individuals lacks empirical evidence.

Our review leads us to propose that there is good evidence supporting a role for RET in maintaining cardiovascular health and again this is likely to be of a comparable magnitude in terms of risk reduction as that seen with AET. Regarding the exercise intensity required to exert beneficial effects on CVD risk factors, evidence demonstrates limited additional benefit to increasing RET intensity. Indeed, low-to-moderate intensity RET (30–69% of 1RM) exerts similar improvements in blood pressure (Cornelissen and Smart, 2013), and blood lipid profiles (Lira et al., 2010; Sheikholeslami Vatani et al., 2011) than high-intensity RET (≥70% of 1RM). Thus, contrary to popular belief, we argue that low-to moderate intensity RET (30–69% of 1RM) is safe and effective even in individuals with CVD or at risk for developing CVD.

RESISTANCE EXERCISE TRAINING AND CANCER

Cancer is a leading cause of morbidity and mortality with approximately 14 million new cases and 9.6 million annual cancer-related deaths worldwide (World Health Organization, Cancer Facts, 2018). Many of these cancer diagnoses share risk factors linked to T2D and CVD and are associated with a sedentary lifestyle (Vainio et al., 2002). In support of this assertion, a wealth of data demonstrate that regular physical activity is associated with a reduced risk of developing cancer, dying from cancer, and improving cancer prognosis (Keum et al., 2016; Moore et al., 2016).

Using data derived from the Health Survey for England and the Scottish Health Survey consisting of 80,000 adults aged >30 years, Stamatakis et al. (2018) demonstrated that adhering to guideline advice to perform RET (at least two times per week) was associated with a 34% reduced risk for cancer mortality; whereas adhering to the AET guidelines provided no statistical benefit. Moreover, cancer survivors who participated in RET at least once per week had a 33% reduction in all-cause mortality (Hardee et al., 2014). A recent comprehensive review conducted by Cormie et al. (2017) demonstrated that regular performance of both RET and AET following the diagnosis of cancer had a protective effect on cancer-specific mortality, cancer recurrence, and all-cause mortality. These observations would be expected, given that muscle mass and strength are inversely associated with cancer mortality (Ruiz et al., 2009; Bennie et al., 2016). Although the aforementioned studies are observational and causation cannot be inferred, together they provide support for the hypothesis that regular performance of RET reduces cancer risk, cancer mortality, and cancer recurrence. Incorporating RET into a combined activity program appears to have complimentary effects on factors related to cancer development.

Dieli-Conwright et al. (2018) demonstrated that following the American College of Sports Medicine/American Cancer Society exercise guidelines for 16 weeks (150 min of AET, and 2–3 sessions of RET/week) in overweight or obese breast cancer survivors improved all components of metabolic syndrome – a comorbid condition prevalent in cancer survivors following treatment that increases the risk for cancer recurrence (Russo et al., 2008) and cancer-specific mortality (Pasanisi et al., 2006). While this work supports the utility of performing both AET and RET in reducing incident and recurrent cancer risk, future randomized controlled trials are warranted to identify which exercise modality (independently) is most effective in this regard.

RET also alleviates patients of some of the unwanted side effects associated with cancer treatment. Current therapeutic approaches (i.e., chemotherapy, radiation therapy, androgen deprivation therapy for prostate cancer) for cancer exacerbate the loss of skeletal muscle mass and strength in patients. Importantly, these adaptions have negative implications for vital clinical endpoints including cancer mortality (Ruiz et al., 2009; Bennie et al., 2016), disease progression, and therapeutic complications (dose-limiting toxicity; Prado et al., 2008). Whole-body, progressive RET (2–4 sets at 60–70% 1RM) can preserve muscle mass and strength in patients with prostate cancer undergoing androgen deprivation therapy (Galvao et al., 2010) or radiation therapy (Segal et al., 2009). A recent meta-analysis in 1200 men with prostate cancer showed that regular RET improved muscular strength, body composition, and 400-m walking performance (Keilani et al., 2017). Importantly, 24 weeks of RET resulted in greater improvements in triglycerides, body

fat, and quality of life than AET (cycle ergometer/treadmill/elliptical for 45 min at 60–75% VO_{2max}) during radiation therapy (Segal et al., 2009). Exciting data from the Supervised Trial of Aerobic Versus Resistance Training (START) trial demonstrated that whole-body, progressive RET (3 sets at 60–70% 1RM) improved lean body mass, strength, fatigue, and chemotherapy completion rate in breast cancer survivors receiving adjuvant treatment, whereas there was no difference between AET (cycle ergometer/treadmill/elliptical for 45 min at 60–80% VO_{2max}) and usual care (Courneya et al., 2007; Adams et al., 2016). In a recent meta-analysis including 11 randomized controlled trials and 1,167 participants (74% women) receiving treatment for various cancers, regular performance of RET led to improvements in lean body mass, strength, and whole-body fat mass (Strasser et al., 2013). These findings are clinically relevant, given that increased adiposity – and the concomitant increase in inflammatory status – is prevalent following cancer treatment and can negatively impact cancer prognosis and increase the risk of recurrence (Vance et al., 2011). Furthermore, the beneficial effects of RET were augmented when RET interventions were of low-to-moderate intensity (≤69% 1RM), which may be more appealing for cancer patients who are unable to – due to comorbidities – lift weights at a high relative intensity (Strasser et al., 2013).

Several biological mechanisms have been proposed to mediate the protective effects of RET on cancer risk and prognosis. RET improves indices of insulin sensitivity, body composition (Strasser et al., 2013), immune function (Hagstrom et al., 2016), sex hormone profile (Ennour-Idrissi et al., 2015; Dieli-Conwright et al., 2018), and markers of inflammation (Strasser et al., 2012; Schmidt et al., 2016; Winters-Stone et al., 2018), all of which are factors hypothesized to contribute to cancer risk and progression (McTiernan, 2008). Recently, skeletal muscle has been recognized as an endocrine organ capable of releasing small peptides into the bloodstream (collectively referred to as myokines), which can exert anti-inflammatory and insulin-sensitizing systemic effects on distant tissues. Given the tight relationship between obesity, insulin resistance, and inflammation with cancer risk and prognosis (Barone et al., 2008; Deng et al., 2016), there is potential for the biological support of exercise-induced myokine secretion in anticancer progression. Exciting data from Pedersen et al. (2016) demonstrate that voluntary wheel running reduced tumor volume by approximately 60% in tumor-bearing C57BL/6 mice. Reductions in tumor volume were associated with natural killer cell infiltration into the tumors, which was dependent upon the release of interleukin-6 (IL-6) from contracting skeletal muscle (Pedersen et al., 2016). In fact, the entire process of IL-6 release from contracting skeletal muscles appeared to be unique as intravenous injections of IL-6 failed to reduce tumor growth (Pedersen et al., 2016). Although the results of Pedersen et al. (2016) demonstrate that contracting skeletal muscles are capable of naturally manufacturing molecules with anti-tumorigenic properties, far less is known regarding the role of RET on myokine release. Given that myokine release in humans is a process dependent upon the contraction of skeletal muscle (Hojman et al., 2018), we hypothesize that RET would lead to a similar increase in myokine secretion as AET. Thus, the relationship between RET and myokine release in combatting malignant tumors warrants investigation.

Similar to AET, there is a role for RET in reducing cancer risk, cancer recurrence, cancer mortality, and improving prognosis during adjuvant therapies. Given that cancer has surpassed CVD as the leading cause of death in several developed countries (Tanday, 2016), these observations are of great importance. Although the importance of RET for breast cancer and prostate cancer is becoming apparent, the effects of RET on other cancer types are equivocal, and warrant further investigation. Future work should be focused upon unraveling the optimal dose, intensity, and mechanisms specific to RET-induced cancer benefits.

RESISTANCE EXERCISE TRAINING RECOMMENDATIONS FOR REDUCING AGE-RELATED CHRONIC DISEASE RISK

The wide-ranging health benefits of regular RET are well established; however, adherence to RET in older adults remains low, and the most commonly cited barriers to participation of RET are: (1) risk of injury (from lifting heavy relative loads) and (2) required access to a gym facility (Burton et al., 2017). However, utilizing one's own body weight as resistance, or light-to-moderate relative loads (30–69% of 1RM) is just as effective as lifting heavy relative loads (≥70% of 1RM) for exerting health benefits (Lira et al., 2010; Lustosa et al., 2011; Sheikholeslami Vatani et al., 2011; Cornelissen and Smart, 2013; Strasser et al., 2013; Csapo and Alegre, 2016; Yang et al., 2017; Stamatakis et al., 2018). Cognizant of these findings, RET recommendations have been formulated, which may aid older adults in adhering to and thus reducing chronic disease risk (**Figure 1**). We suggest that exercise volume is more salient than exercise intensity in mediating

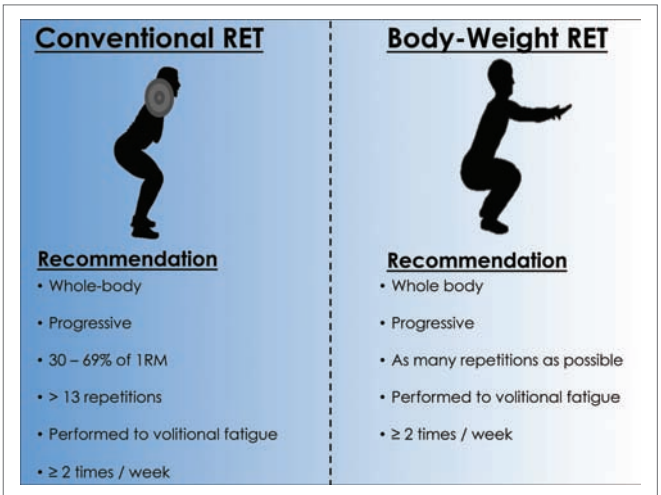

FIGURE 1 | Evidence-based resistance exercise training recommendations for reducing age-related chronic disease risk. Abbreviations: 1RM, 1 repetition maximum.

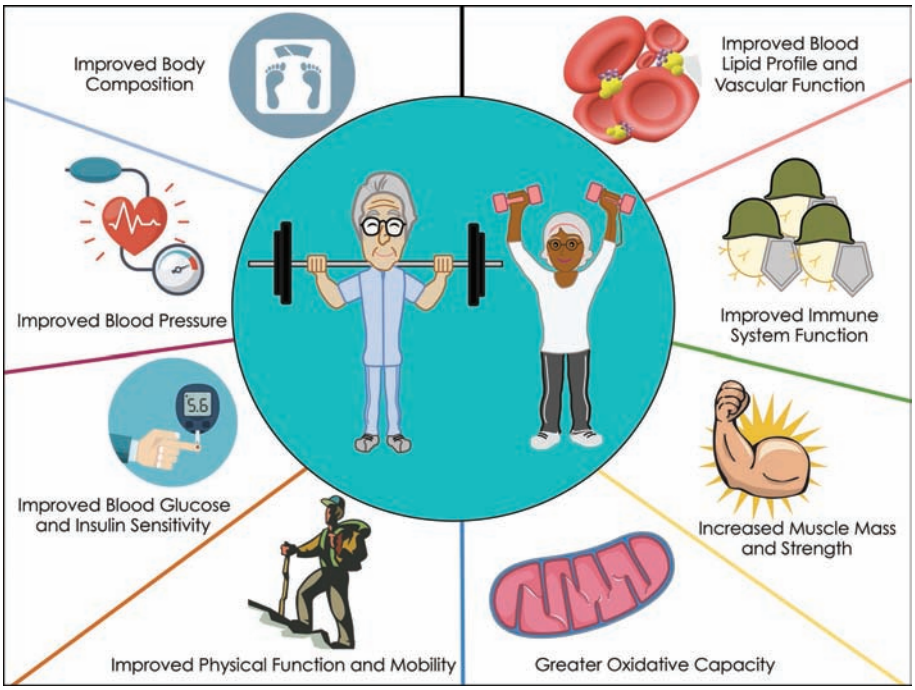

FIGURE 2 | Proposed mechanisms whereby RET influences chronic disease risk.

TABLE 1 | Age-related changes in risk factors for chronic disease and the adaptive responses to aerobic exercise training and resistance exercise training.

Adaptations	Aging	AET	RET
Whole-body adaptations			
Muscle strength	↓	↔ (Grgic et al., 2018)	↑↑ (Cadore et al., 2013)
Muscle mass	↓	↔ (Grgic et al., 2018)	↑↑ (Peterson et al., 2011)
Bone mineral density	↓	↔ (Villareal et al., 2017)	↑ (Villareal et al., 2017)
VO_{2peak}	↓	↑↑ (Bienso et al., 2015)	↑ (Bienso et al., 2015)
Physical function	↓	↑ (Jadczak et al., 2018)	↑ (Jadczak et al., 2018)
Type II diabetes			
Risk	↑	↓ (Knowler et al., 2002)	↓ (Grontved et al., 2012)
Glycemic control	↓	↑ (Bienso et al., 2015)	↑ (Bienso et al., 2015)
Insulin signaling	↓	↑ (Bienso et al., 2015)	↑ (Bienso et al., 2015)
Oxidative capacity	↓	↑ (Jubrias et al., 2001)	↑ (Jubrias et al., 2001)
Cardiovascular disease			
Risk	↑	↓ (Tanasescu et al., 2002)	↓ (Tanasescu et al., 2002)
Blood pressure	↑	↔ (Cornelissen and Smart, 2013)	↓ (Cornelissen and Smart, 2013)
Blood lipids			
High-density lipoprotein	↓	↑ (Yang et al., 2014)	↑ (Yang et al., 2014)
Low-density lipoprotein	↑	↓ (Yang et al., 2014)	↓ (Yang et al., 2014)
Cholesterol	↑	↓ (Yang et al., 2014)	↓ (Yang et al., 2014)
Triglycerides	↑	↓ (Yang et al., 2014)	↓ (Yang et al., 2014)
Cancer			
Incident Risk	↑	↓ (Keum et al., 2016)	↓ (Keum et al., 2016)
Risk of recurrence	↑	↓ (Dieli-Conwright et al., 2018)	↓ (Dieli-Conwright et al., 2018)
Quality of life	N/A	↑ (Segal et al., 2009)	↑↑ (Segal et al., 2009)
Therapy completion rate	N/A	↔ (Courneya et al., 2007)	↑ (Courneya et al., 2007)
Immune function	↓	↑ (McTiernan, 2008)	↑ (Hagstrom et al., 2016)
Inflammation	↑	↓ (McTiernan, 2008)	↓ (Strasser et al., 2012)

↑ Indicates an increasing effect on the parameter, ↓ indicates a decreasing effect on the parameter, and ↔ indicates no effect on the parameter. The number of arrows denotes the magnitude of effect. Abbreviations: N/A, not available.

the positive adaptations discussed herein, and that as long as RET is performed to volitional fatigue, older adults can reap the health benefits of RET.

RESISTANCE EXERCISE TRAINING: FROM A SUPPORTING TO A STARRING ROLE?

The evidence presented in this review demonstrates the beneficial effects of RET on reducing chronic disease risk (mobility disability, T2D, CVD, and cancer) in older adults (**Figure 2**). Regular performance of RET improves muscle mass, strength, and function, and can have direct effects on the primary prevention of a number of chronic diseases. On the basis of the evidence we have highlighted, RET-induced benefits in chronic disease risk are equivalent if not superior in magnitude as AET (**Table 1**). Nonetheless, a number of agencies endorse performing 150 min of AET per week to mitigate age-related chronic disease risk, whereas the role of RET on overall health is typically underappreciated. Furthermore, only 2.4% of older adults achieve this AET recommendation (Troiano et al., 2008), and this may be due in part to the guidelines including intensities or volumes potentially unreachable for older adults limited by many comorbidities. Based on the evidence presented in this narrative review, we propose that RET may serve as *"another tool in the toolbox"* for older adults to remain physically active and combat chronic disease risk. We do acknowledge that some knowledge gaps exist such as the optimal dose and intensity of RET required to exert health benefits and clinical trial evidence showing head-to-head comparisons with AET, and further investigations are needed.

AUTHOR CONTRIBUTIONS

JCM, TS, and SMP wrote the initial draft of the manuscript. All authors edited and approved the final version of the manuscript and agree to be accountable for all aspects of the work. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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Combined Resistance and Stretching Exercise Training Benefits Stair Descent Biomechanics in Older Adults

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Introduction: Stair descent is a physically demanding activity of daily life and common risk for falls. Age-related deteriorations in ankle joint capacities make stair descent particularly challenging for older adults in built environments, where larger rise steps are encountered. Exercise training may allow older adults to safely cope with the high biomechanical demands of stair descent. However, little is known about the demands of increased rise stairs for older adults, nor the impact of exercise.

Aim: We investigated whether the effects of lower-limb resistance training would alter joint kinetics and movement strategies for older adults when descending standard rise, and increased rise stairs.

Methods: Fifteen older adults descended a four-step stair adjusted to standard rise (170 mm), and increased rise (255 mm) on separate visits. Between these two visits, randomly allocated participants underwent 16 weeks of either: resistance exercise training ($n = 8$) or habitual activity ($n = 7$). Kinetic data were measured from step-mounted force plates, and kinematic data from motion-capture cameras. Training involved twice-weekly sessions of lower-limb resistance exercises (three sets of ∼8 repetitions at ∼80% three-repetition maximum), and static plantarflexor stretching (three, 45 s holds per leg).

Results: *Standard stairs* – Peak ankle joint moments increased ($p < 0.002$) and knee joint moments decreased ($p < 0.01$) during descent after exercise training. Peak centre of pressure-centre of mass (CoP-CoM) separations increased in posterior ($p = 0.005$) and medio-lateral directions ($p = 0.04$) after exercise training. Exercise training did not affect CoM descent velocity or acceleration. *Increased rise stairs* – Required greater ankle, knee, and hip moments ($p < 0.001$), peak downward CoM velocity and acceleration ($p = 0.0001$), and anterior-posterior CoP-CoM separation ($p = 0.0001$), but lower medial-lateral CoP-CoM separation ($p < 0.05$), when compared to standard stair descent. Exercise training did not affect joint kinetics or movement strategies.

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Discussion: Exercise training increased the maximum joint ROM, strength and force production of the ankle, and enabled a greater ankle joint moment to be produced in single-leg support (lowering phase) during standard stair descent. Descending increased rise stairs raised the task demand; exercise training could not overcome this. Future research should prioritize the ankle joint in stair descent, particularly targeting plantarflexor torque development across stairs of varying riser heights.

Keywords: stair negotiation, joint moments, stability, stretching, aging, movement control

INTRODUCTION

Stair negotiation is a challenging activity of daily living that people perform in both home and public environments. Stair descent presents a particular challenge, in that the individual must control the lowering of body mass in single-limb support, whilst the contralateral limb moves to the step below. This task involves lower limb joint ranges of motion and moments exceeding those required for level over-ground gait (McFadyen and Winter, 1988; Riener et al., 2002), and presents significant challenge to dynamic balance (Zachazewski et al., 1993). Adequate neuromuscular control is required to ensure that the swinging advancing foot negotiates the step edge bearing the loaded limb, as it moves forward and downward, and is then safely placed on the lower step. Muscular control at the ankle joint becomes critically important during landing as the ground reaction forces produced on step contact are dissipated in the landing-limb (Riener et al., 2002; Reeves et al., 2008a; Buckley et al., 2013). Muscle-tendon units undergo eccentric contractions in stair descent to decelerate body segments and absorb mechanical work. These further increase task demand for older people, who operate closer to their limits of eccentric ankle strength and dorsiflexion range, in comparison to younger people (Reeves et al., 2008a). As a coping strategy to meet this increased demand, older adults appear to redistribute joint moments, by maintaining knee joint moment (around 42% maximal), whilst lowering ankle joint moment within safer ranges (around 75% maximal) (Reeves et al., 2009), to operate within maximal capacities and reduce potential falls risk.

Falls incidence increases with age and is influenced by involvement in demanding daily activities. For example, 50% of adults aged 65 years and over, and 80% of adults aged 80 years and over, fall on average once a year (American Geriatrics Society et al., 2001), with the majority of falls occurring on stairs (Svanstrom, 1974; Hemenway et al., 1994). Previous work documenting age-related adaptations during stair descent have revealed valuable insights into potential biomechanical factors contributing to an increased falls risk in older adults (Buckley et al., 2013; Novak et al., 2016; Dixon et al., 2018). Older people operate at higher maximum eccentric ankle capacities and joint ranges than the young (Reeves et al., 2008a), which leaves little reserve capacity for the old to cope with unanticipated perturbations occurring in stair descent.

To lower the centre of mass (CoM) to the step below, the lower-limb eccentrically flexes under the control exerted by the joint moments produced. If the joint moments are not sufficiently high, then the lowering velocity may become too excessive. Older adults adopt conservative strategies to safely negotiate these demands in stair descent, notably reducing peak CoM and advancing limb downward velocities (Buckley et al., 2013). Age-related deterioration in eccentric ankle force-generating capacity would reduce the ability of older people to absorb an increased downward velocity (momentum) during landing.

Exercise training interventions may be important in supporting older adults to meet the biomechanical demands of stair descent. Resistance exercise training has been shown to improve maximal lower-limb function and mobility (Bean et al., 2004) and stair negotiation performance (Capodaglio et al., 2005) of healthy adults aged over 70 years. For overground walking, lower-limb stretching training appears effective in increasing hip extension motion and gait stride length after 10 weeks (Watt et al., 2011), whilst ankle plantarflexor stretching has been shown to increase range of motion, step length and step velocity after 4 weeks (Cristopoliski et al., 2009). However, even long-term (i.e., 12 months) combined resistance and aerobic exercise training may have scant impact on the gait speed and joint motions of older adults in stair descent (Mian et al., 2007), despite improvements in lower-limb muscle mass, strength, and power (Morse et al., 2005). Where others have combined exercises, none have adopted training programs that are targeted to the impaired muscle groups of the lower-limbs (e.g., ankle joint motion and strength), nor assessed the joint kinetics or movement strategies of older adults when descending stairs. A specific, combined strengthening (to better cope with the high joint moment demand) and stretching (to better cope with the high ankle dorsiflexion demand) program for the ankle and knee muscle groups is therefore necessary.

Potential adaptations conferred by exercise interventions to how stairs are negotiated should be studied across different stair-riser heights, as stairs and steps encountered in daily life can vary from those that are within national regulatory guidelines [e.g., maximum permitted stair riser for individual dwellings in the United Kingdom is 220 mm; (HM Government, 2013)], to those that are higher, as is the case for low-floor public transport vehicles (Institute for Transportation, and Development Policy [ITDP], 2018) and unregulated stairs and steps.

Even for young adults using the traditional step-over-step gait pattern, descending steps with risers increased by 50% (i.e., 255 mm) increases peak ankle (28.6%) and knee joint moments (29.8%), when compared to descending standard rise stairs (i.e., 170 mm) (Spanjaard et al., 2008). Descending stairs with increased rise increases joint moments and the challenge to

balance, as individuals must step downward over greater vertical distance, and consequently generate greater joint moments to dissipate ground reaction forces and arrest CoM downward velocity. When adopting the step-over-step strategy for increased rise stair descent, older adults place greater demand on the ankle plantarflexors, whilst reducing knee extensor demand during landing (King et al., 2018). This presents further falls risk given older adults descend stairs closer to their ankle joint biomechanical limits. The biomechanical demands of stair descent are not known for older adults on increased rise steps, nor are the potential effects of exercise training. This is important as older adults operate closer to their maximal capacities, than the young.

It seems then appropriate to (i) assess whether exercise training can affect the locomotion of older adults when descending standard stairs, (ii) quantify the demands of descending increased rise stairs for older adults, and finally (iii) assess whether exercise training can reduce any additional demands presented by descending increased rise stairs in older adults. The main aims for the present study were divided to focus on standard stairs and increased riser stairs as follows:

(1) To investigate whether 16 weeks of lower-limb resistance and stretching exercise training would lead to an alteration in joint kinetics, and movement strategies in older adults when descending standard stairs.

(2) To determine whether descending stairs of increased riser height modifies joint kinetics and movement strategies in older adults, and whether exercise training can alter these biomechanics.

MATERIALS AND METHODS

Participants

Fifteen older adults (eleven women, four men; mean ± SD; age, 75 ± 3 years; height, 1.62 ± 0.07 m; body mass, 69.3 ± 11.1 kg) from the local community provided written informed consent to participate in the study. Participants were then randomly allocated to exercise and control groups. The research protocol was approved of by the ethics committee of the Manchester Metropolitan University. All participants were free from recent musculoskeletal and neuromuscular injury that would influence gait.

Experimental Protocol

Stair descent trials were performed on a four-step stair, with steps of 280 mm going (tread) and 900 mm width and adjustable to standard rise (170 mm), and 50% increased rise (255 mm). For reference, the top step below the landing was labeled as step one, and the floor at the stair base as step four (left limb contacting steps two and four; right limb contacting steps one and three). Eight participants (age, 75 ± 4 years; height, 1.62 ± 0.09 m; body mass, 69.7 ± 12.3 kg) negotiated standard rise and increased rise stairs in a counter-balanced order, on separate visits, before and after 16 weeks of resistance exercise and stretching training (twice weekly). A non-training, control group consisting of seven participants (age, 75 ± 2 years; height, 1.63 ± 0.06 m; body mass, 71.2 ± 11.5 kg) underwent stair testing before, and after the same period of time as the exercise intervention (control period), whilst continuing with their habitual activity. Random allocation was used to assign participants to the respective groups. Three trials were performed on each visit.

On each visit, participants walked down stairs bare-feet. Following familiarization trials, participants stood at the top of the stairs and, leading with the right leg, they were asked to walk down the stairs unaided, at a self-selected pace, in a step-over-step manner. Kinematic and kinetic analysis focussed on a single gait cycle for the left limb in steady-state, from the first touch-down onto step one, to the second touch-down onto step four (force plate on the floor), and subsequently averaged across the three trials. Gait cycles refer to the left leg, defined by the events of: initial foot contact (step two), single-leg stance (step two), double support, foot off (step two), and final foot contact with the step below (step four) (Nadeau et al., 2003; Reeves et al., 2008a). Single support represents the proportion of the gait cycle when the participant is supported by the left leg; double support represents support by both legs.

Kinetic and Kinematic Data

Three piezoelectric force plates were mounted in each step (Kistler type Z17068, Kistler Instruments, Winterthur, Switzerland); the steel steps were bolted individually to the laboratory floor. One additional force plate was mounted in the concrete floor at the bottom of the stair (Kistler type 9253A, Kistler Instruments, Winterthur, Switzerland). Kinetic data were collected at 1,080 Hz, down-sampled to 120 Hz, and subsequently analyzed in anterior-posterior (sagittal plane), and medial-lateral (frontal plane) directions. Ground reaction forces were measured independently for each plate.

Nine motion-capture cameras (VICON 612 system, VICON Motion Systems Ltd., Oxford, United Kingdom) recorded the displacement of retro-reflective markers whilst the participants performed stair descent trials. Thirty-four markers were placed onto anatomical landmarks as recommended by the Helen Hayes plug-in-gait marker set. Markers were secured with double-sided, adhesive tape to the skin, or to tight-fitting shorts and t-shirt. Segmental motion data were sampled at 120 Hz. Captured descent trials were processed using Workstation software using participant anthropometric measures and the "plug-in-gait" model (VICON Motion Systems Ltd., Oxford, United Kingdom). For processing, gap filling was applied to marker trajectories with less than 10 missing samples; for 10 or more, trials were excluded. A Woltring filter was then applied (mean square error value, 20) to ensure constant treatment across the data-set. Finally, joint kinematics and kinetics were processed by running inverse kinematics and inverse dynamics analyses (static and dynamic Plug-in-Gait model) in the Workstation software. Kinematic and kinetic data for the ankle, knee, and hip joints, alongside toe, heel and CoM co-ordinates (x, y, z), were exported to ASCII format for further analysis. Data were analyzed in the sagittal plane for the ankle, knee and hip joints, and in the frontal plane for the hip joint according to previous observations (Nadeau et al., 2003; Mian et al., 2007).

Maximum Functional Capacities

Eccentric, maximum voluntary contractions of the knee extensors and plantarflexors were assessed at baseline and 16 weeks later, for exercise training and control groups using an isokinetic dynamometer (Cybex NORM, New York, NY, United States). Using the left leg, participants performed three maximum contractions each (with 2 to 3 min rest), at angular velocities of: 60, 120, 180, and 240°·s^{-1}. Knee extensions were performed seated, with the hip at 85° (hip supine = 0°); ankle plantarflexions were performed lying prone, with the knee at 0° (full extension) (Reeves et al., 2008a). Isometric, maximum voluntary contractions of the plantarflexors were assessed to determine the rate of torque development (Reeves et al., 2003); instruction was given to perform each contraction (lasting approximately 1 to 2 s) as rapidly as possible (Aagaard et al., 2002).

Maximum assisted dorsiflexion angle was also determined in the prone position with the knee extended to assess the impact of the stretching intervention on maximum dorsiflexion range of motion. With the left foot attached to the footplate, the investigator manually dorsiflexed the foot until the participant expressed they could no longer tolerate any further dorsiflexion movement.

Joint Moment Normalization

Ankle and knee joint moments produced in stair descent were normalized relative to maximum capacities for each participant, as follows: (i) maximal eccentric extensor moments were quantified from the left leg across four different angular velocities (see section "Maximum functional capacities" above), (ii) the maximum joint moment in the gait cycle (i.e., the point of highest demand) and it's corresponding angular velocity were then identified for each respective joint, and (iii) the angular velocity at maximum moment for gait was matched, to the most closely corresponding angular velocity for dynamometry-based measurements (Reeves et al., 2008a). Finally, the maximum moment in the gait cycle was divided by the dynamometry-based maximum moment for the corresponding angular velocity.

Joint power was determined for each lower limb joint throughout the gait cycle (unpublished data), as the product of joint moment and joint angular velocity. Joint mechanical work was computed by integrating the joint power curves of the ankle, knee and hip joints, respectively, using the trapezium method. Joint work was expressed as positive or negative to indicate when a joint was operating eccentrically (i.e., negative work) or concentrically (i.e., positive work). Positive work indicates energy production, whereas negative work indicates energy absorption.

Center of Mass Calculations: Displacement, Velocity, and Acceleration

The centre of pressure-centre of mass (CoP-CoM) separation was characterized as the difference between the projections of the CoP of the ground reaction force vector (x, y, z), and CoM, for both sagittal and frontal planes. For trials where a participant had one foot on two separate force plates, a weighted average was calculated for CoP (Reeves et al., 2008b). Minimum and maximum values of CoP-CoM separation were used to represent anterior, and posterior separation in the sagittal plane, and medial and lateral in the frontal plane during each gait cycle.

Center of mass velocity and acceleration were determined following previous methods (Buckley et al., 2013), for the initial step down (i.e., transition from stair landing to step one) divided into descent and landing phases. Movement initiation was determined from when the vertical velocity of the lead limb's heel marker first exceeded 0.05 m/s in the upward direction, for six consecutive frames (sampling rate, 120 Hz; time span, 0.01 s), whereas the subsequent foot contact (on to step one) was identified as when the vertical ground reaction force exceeded 20 N (descent phase). The landing phase was from foot contact, to the point at which downward CoM velocity reduced to zero or became positive. The following characteristics were identified for the initial step down: peak foot (i.e., heel) velocity, CoM peak downward velocity, CoM peak acceleration (descent phase), and CoM peak acceleration (landing phase).

Resistance and Stretching Exercise Training

The participants undergoing exercise training performed small group sessions involving supervised resistance and stretching exercises for the lower-extremities twice a week, for 16 weeks. Resistance exercises were conducted on leg-press, knee extension and calf-press machines, with the three-repetition maximum (3RM) determined during session one. A standardized warm up (15 repetitions at 40% 3RM) commenced each exercise, which, following a short rest, involved three sets of ∼8 repetitions (75 to 80% 3RM). For the calf-press, three sets of 10 to 12 maximal, isometric contractions (1 to 2 s duration each, performed as rapidly as possible) were also performed to improve plantarflexor rate of torque development. The 3RM was reassessed every 4 weeks to monitor progression of exercise training load (Welle et al., 1996; Mian et al., 2007).

Plantarflexor muscles underwent static stretching, one leg at a time, prior to resistance exercises. Standing with the stretched leg extended on wedges inclined at 15 or 25° (Han et al., 2014). Stretches were held for 45 s, with three repetitions per leg. Intensity was raised by: (1) maintaining the stretched leg in extension, whilst shifting the supporting leg forward; and/or (2) increasing wedge incline from 15 to 25°. Stretching was performed to increase maximum dorsiflexion angle, because stair descent requires application of large dorsiflexion support moments, at a large dorsiflexion angle (Reeves et al., 2008a), far exceeding those during stair ascent (Protopapadaki et al., 2007; Reeves et al., 2009).

Statistical Analysis

Two-way, mixed model ANOVAs [time (pre, post 16 weeks) (within-subject factor) × group (exercise, control) (between-subject factor)] were performed separately for standard and increased riser stairs to test the effects of (i) exercise training and (ii) step rise height, on the temporal-spatial characteristics, joint moments, and CoM parameters during stair descent at

0 and 16 weeks. Bonferroni adjustments were used to identify specific training effects for each group. Data analysis was performed using IBM SPSS Statistics Version 21 (IBM Corp, Armonk, NY, United States), with statistical significance accepted as $p < 0.05$.

RESULTS

Maximum Functional Capabilities

Maximum eccentric knee torques assessed on a dynamometer increased at all angular velocities after exercise training (**Table 1**), whereas maximum eccentric ankle torque increased only at $60°{\cdot}s^{-1}$. The angles of peak torque were not different for knee and ankle joints after exercise training ($p > 0.05$). For range of motion, maximum assisted dorsiflexion angle increased by 10.8% after exercise training ($p = 0.03$). Maximum isometric, rate of torque development increased after exercise training for both joints (knee 47.8%; ankle 21.7%; $p < 0.05$).

Standard Rise Stair Descent

Temporal-Spatial Characteristics

No accidents (e.g., slips, oversteps, or falls) occurred during descent trials of standard or increased rise stairs. Resistance exercise training did not affect gait characteristics during standard stair descent (stride frequency: 79.9 ± 15.6 steps/min, step length: 0.35 ± 0.03 m; single support duration: 46.0 ± 5.5%; double support duration: 25.6 ± 6.8%).

Joint Moments (Absolute)

Peak ankle joint moments during both the single-leg support (lowering phase) ($F_{2,8} = 6.25$, $p = 0.02$, $d = 0.33$) and double support (landing phase) ($F_{2,8} = 6.1$, $p = 0.027$, $d = 0.32$) increased after exercise training (**Figure 1** and **Table 2**). Peak knee joint extension moment decreased after exercise training (from the lowering phase) ($F_{2,8} = 28.8$, $p < 0.01$, $d = 0.69$; **Table 3**). Hip joint flexion moment peaks (during the swing phase, from single-leg stance to final left foot off; $p < 0.05$; **Table 4**) increased in the swinging limb after exercise training.

Joint Moments (Normalized)

Normalized ankle joint moments were affected by exercise ($F_{2,8} = 11.3$, $p = 0.005$; $d = 0.46$). Normalized ankle moment increased (pre, 0.89 ± 0.17; post, 1.39 ± 0.63), whereas knee joint moment decreased ($F_{2,8} = 39.2$, $p = 0.0001$; $d = 0.75$) (pre, 1.03 ± 0.38; post, 0.67 ± 0.26) after exercise training.

Negative Work

Exercise training did not affect ankle or hip joint negative work when descending standard stairs ($p > 0.48$). Knee joint work on standard stairs decreased after training ($p = 0.05$; **Figure 2**). Total leg work decreased from before (−2.12 ± 0.11 J) to after training (−1.81 ± 0.13 J) when descending standard stairs ($p = 0.03$, $d = 0.59$).

Centre of Pressure (CoP)-Centre of Mass (CoM) Separation

There was no difference in total excursion of the anterior-posterior CoP-CoM separation after exercise training (**Figure 3** and **Table 5**). Peak CoP-CoM separation in posterior direction (CoM behind the CoP) ($F_{2,8} = 11.75$; $p = 0.005$; $d = 0.5$, difference 21 mm) increased after exercise training.

Total excursion of the medial-lateral CoP-CoM separation was greater after exercise training ($F_{2,8} = 5.5$; $p = 0.04$; $d = 0.3$, mean difference 48.8 mm).

Centre of Mass (CoM) Velocity and Acceleration

Exercise training did not affect the CoM velocity and CoM acceleration when descending standard stairs (**Table 6**).

Increased Rise Stair Descent

Temporal-Spatial Characteristics

Step rise height did affect gait characteristics during stair descent with increased rise stairs, requiring lower stride

TABLE 1 | Maximum functional capabilities of resistance exercising and non-exercising control older adults.

		Exercise training group (n = 8)		Control group (n = 7)	
	Angular velocity ($°{\cdot}s^{-1}$)	Pre	Post	Pre	Post
Maximum knee peak torque (N·m)	240	52.9 ± 28.5	72.4 ± 35.3**	70.2 ± 27.6	74.9 ± 31.1
	180	64.8 ± 38.6	92.8 ± 36.6**	94.5 ± 42.4	104.2 ± 25.1
	120	78.5 ± 38.3	106.0 ± 42.6*	104.1 ± 41.3	120.1 ± 22.0
	60	88.2 ± 35.8	114.4 ± 43.1**	117.1 ± 30.6	123.6 ± 32.0
Maximum ankle peak torque (N·m)					
	240	52.7 ± 14.4	58.8 ± 28.0	60.6 ± 22.5	56.8 ± 18.8
	180	71.9 ± 32.6	79.1 ± 31.5	82.3 ± 22.9	81.0 ± 26.7
	120	79.3 ± 36.6	103.9 ± 38.9	104.2 ± 29.7	100.0 ± 26.8
	60	86.3 ± 32.5	116.7 ± 35.0**	125.6 ± 37.1	108.1 ± 22.5*
Rate of knee torque development ($N{\cdot}m{\cdot}s^{-1}$)		478.0 ± 255.4	706.4 ± 244.5*	407.3 ± 225.9	310.1 ± 240.3
Rate of ankle torque development ($N{\cdot}m{\cdot}s^{-1}$)		324.6 ± 142.5	395.2 ± 131.5*	270.5 ± 98.3	327.1 ± 88.8
Maximum assisted dorsiflexion angle (°)		33.3 ± 4.3	36.9 ± 3.1*	33.6 ± 1.8	35.6 ± 3.1

*Values are mean ± SD. Significant pre-post training difference, *$p < 0.05$, **$p < 0.01$. Data correspond to the right limb.*

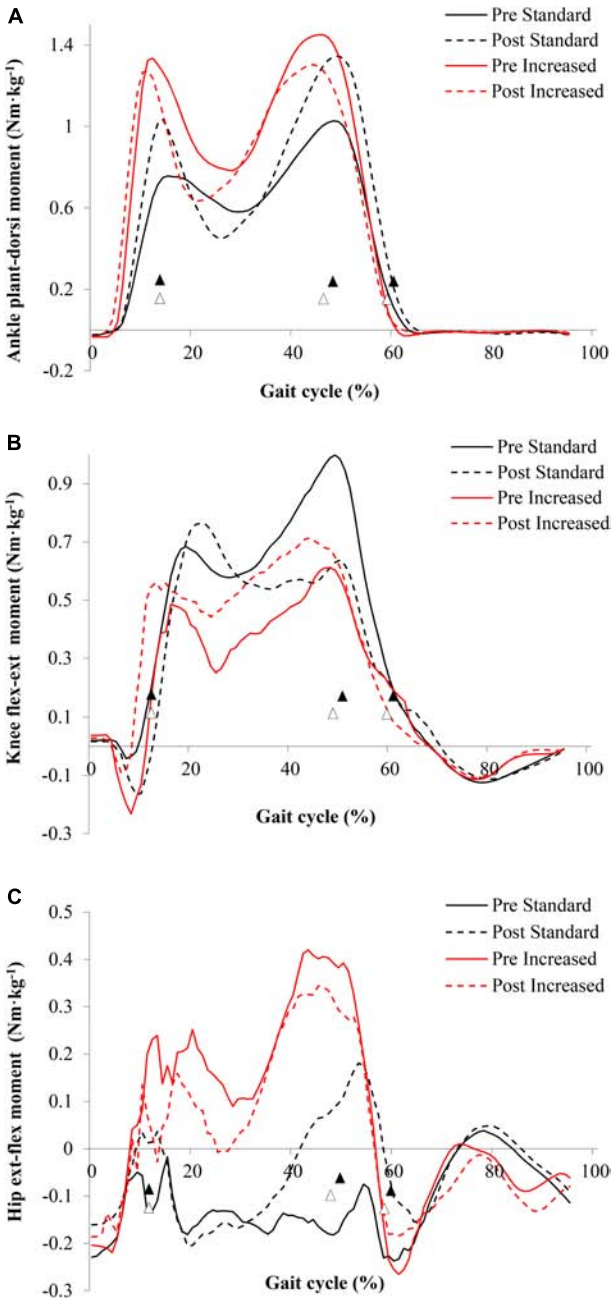

FIGURE 1 | Peak joint moments of older adults descending stairs of different dimensions before and after 16 weeks of exercise training for the ankle **(A)**, knee **(B)**, and hip **(C)** joints. Gait cycle events (▲ standard, △ increased) refer to initial foot contact (0%), single-leg stance, double support, foot off, and final foot contact with the below step (100%); data correspond to the left limb. Values are mean (n = 8).

frequency (67.9 ± 9.6 steps/min, p = 0.001), longer step length (0.40 ± 0.04 m, p = 0.001) and prolonged single support (57.3 ± 8.3%, p = 0.001), but similar double support (20.7 ± 8.2%, p > 0.05). Exercise training reduced stride frequency when descending increased rise stairs (61.0 ± 13.5 steps/min, p = 0.04).

Joint Moments (Absolute)

When compared to standard stairs descending increased rise stairs required greater ankle, knee and hip joint moments (p < 0.001; **Tables 2–4**), greater knee flexion moments during both lowering and landing phases (p = 0.004), but reduced knee extension moments during landing (p < 0.001).

Peak ankle joint moments during landing showed a tendency to decrease after exercise training (p = 0.06; d = 0.46), whereas peak knee joint extension moments during lowering increased after exercise training (p = 0.06; d = 0.37). Hip joint moments were unaffected by exercise training.

Joint Moments (Normalized)

Normalized ankle joint moments were affected by stair dimension ($F_{2,8}$ = 11.3, p = 0.005; d = 0.46). Normalized ankle and knee joint moments were greater when descending increased rise stairs (p < 0.001; **Tables 2**, **3**), when compared to standard stairs.

Normalized ankle and knee joint moments when descending increased rise stairs were unaffected by exercise training (p > 0.4).

Negative Work

Descending increased rise stairs required greater ankle (rise effect, p = 0.0001) and hip joint work (rise effect, p = 0.0001), when compared to standard stair descent. In addition, total leg work was greater descending increased rise stairs (−2.58 ± 0.35 J, p = 0.02; d = 0.34), when compared to descending standard stairs (−2.09 ± 0.16 J).

Exercise training had no effect on the amount of negative joint work at the ankle, knee or hip joint, when descending increased rise stairs (p > 0.48).

Centre of Pressure (CoP)-Centre of Mass (CoM) Separation

Descending stairs of increased rise involved greater CoP-CoM separation in anterior-posterior ($F_{2,8}$ = 7.0; p = 0.02; d = 0.35) and medial-lateral directions ($F_{2,8}$ = 23.43; p = 0.0001; d = 0.64), when compared to standard stairs (**Figures 3C,D**).

Increased rise stair descent involved greater peak separation in the posterior direction (CoM behind CoP) ($F_{2,8}$ = 24.1; p = 0.0001; d = 0.67) and lesser separation in the anterior direction (CoM in-front of CoP) ($F_{2,8}$ = 14.23; p = 0.002; d = 0.52), when compared to standard stair descent.

Exercise training did not affect the total excursion of the CoP-CoM separation in anterior-posterior and medial-lateral directions (p > 0.02) when descending increased rise stairs (**Figure 3**).

Centre of Mass (CoM) Peak Velocity and Acceleration

Acceleration (rise effect, p = 0.0001; time effect, p = 0.003), and lead foot velocity (rise effect, p = 0.002) (p < 0.002; **Table 6**), when compared to descending standard stairs.

Exercise training did not affect the CoM velocity and CoM acceleration when descending stairs of increased rise (p > 0.1). However, the control group's CoM peak downward acceleration was slightly greater in the descent phase (from −3.7 m/s^2 to

TABLE 2 | Ankle joint moments of older adults descending stairs of different dimensions before and after 16 weeks of exercise training.

	Exercise group (n = 8)		Control group (n = 7)		
Gait cycle events	**Pre**	**Post**	**Pre**	**Post**	***p*-value**
Standard stairs					
Initial foot contact (0%)	−0.03 ± 0.01	−0.02 ± 0.01	−0.02 ± 0.001	−0.02 ± − 0.01	0.19
Single-leg stance	0.65 ± 0.14	0.97 ± 0.25*	0.67 ± 0.19	0.86 ± 0.33	0.02
Double support	1.02 ± 0.20	1.35 ± 0.26*	1.04 ± 0.10	1.16 ± 0.16	0.027
Foot off	0.07 ± 0.16	0.13 ± 0.24	0.09 ± 0.07	0.12 ± 0.19	0.65
Final foot contact (100%)	−0.01 ± 0.01	−0.02 ± 0.00	−0.025 ± 0.02	0.11 ± 0.30	0.25
Normalized maximum ankle moment (%)	0.89 ± 0.17	1.39 ± 0.63*	0.91 ± 0.35	1.06 ± 0.18	0.006
Increased rise stairs					
Initial foot contact (0%)	−0.03 ± 0.02	−0.03 ± 0.01	−0.03 ± 0.001	−0.03 ± 0.001	0.21
Single-leg stance	1.34 ± 0.34†	1.32 ± 0.39	1.45 ± 0.57	1.25 ± 0.35	0.44
Double support	1.35 ± 0.22†	1.13 ± 0.29*	1.42 ± 0.23	1.33 ± 0.19	0.021
Foot off	−0.01 ± 0.03†	0.01 ± 0.07	0.09 ± 0.16	0.08 ± 0.16	0.95
Final foot contact (100%)	−0.02 ± 0.01	−0.01 ± 0.01	−0.014 ± 0.001	−0.02 ± 0.03	0.18
Normalized maximum ankle moment (%)	1.75 ± 0.59†	1.78 ± 0.51	1.41 ± 0.58	1.54 ± 0.42	0.58

*Values are mean ± SD. Significant difference: training *, step dimension* †$p < 0.05$.

TABLE 3 | Knee joint moments of older adults descending stairs of different dimensions before and after 16 weeks of exercise training.

	Exercise group (n = 8)		Control group (n = 7)		
Gait cycle events	**Pre**	**Post**	**Pre**	**Post**	***p*-value**
Standard stairs					
Initial foot contact (0%)	0.02 ± 0.03	0.02 ± 0.02	−0.01 ± 0.04	−0.004 ± 0.02	0.34
Single-leg stance	0.20 ± 0.1	−0.03 ± 0.34*	0.09 ± 0.10	−0.08 ± 0.22	0.02
Double support	1.00 ± 0.17	0.63 ± 0.25*	0.75 ± 0.19	1.07 ± 0.29	0.0001
Foot off	0.18 ± 0.12	0.17 ± 0.05	0.28 ± 0.10	0.24 ± 0.19	0.78
Final foot contact (100%)	−0.02 ± 0.03	−0.02 ± 0.03	−0.02 ± 0.04	−0.05 ± 0.04	0.68
Normalized maximum knee moment (%)	1.03 ± 0.48	0.67 ± 0.26*	0.75 ± 0.25	0.66 ± 0.26	0.01
Increased rise stairs					
Initial foot contact (0%)	0.04 ± 0.03	0.03 ± 0.03	0.01 ± 0.03	−0.01 ± 0.01	0.76
Single-leg stance	0.16 ± 0.41†	0.66 ± 0.37*	0.28 ± 0.40	0.33 ± 0.25	0.06
Double support	0.60 ± 0.24†	0.65 ± 0.29	0.50 ± 0.23	0.52 ± 0.24	0.82
Foot off	0.19 ± 0.11	0.08 ± 0.09	0.14 ± 0.11	0.12 ± 0.12	0.11
Final foot contact (100%)	−0.02 ± 0.04	−0.01 ± 0.02	−0.04 ± 0.03	−0.04 ± 0.03	0.27
Normalized maximum knee moment (%)	0.76 ± 0.50†	0.71 ± 0.33	0.70 ± 0.28	0.68 ± 0.22	0.58

*Values are mean ± SD. Significant difference: training *, step dimension* †$p < 0.05$.

-4.4 m/s^2; $p = 0.04$; $d = 0.28$) on increased rise stairs after 16 weeks of not training.

DISCUSSION

The study describes the lower-limb joint kinetics and the CoM motion of older adults descending stairs of different step dimensions before, and after 16 weeks of resistance exercise training. The exercise training, which included plantarflexor stretching, improved ankle torque (at 60°·s^{-1}) and knee torque (from 60 to 240°·s^{-1}), and maximum dorsiflexion joint range when assessed by isokinetic dynamometry. Importantly, exercise had a positive effect of redistributing joint moments in standard rise stair descent, specifically by allowing a greater ankle and hip moment, whilst reducing knee moments. This redistribution of joint moments after exercise was linked with a balance control strategy that could be regarded as safer, by maintaining the CoM further behind the CoP during stair descent. Increasing stair rise by 50% required the participants to take longer steps. This resulted in prolonged single-limb support, increased lower-limb joint moments, increased downward acceleration of the CoM and presented further challenge to (anterior-posterior) postural stability, when compared to standard stair descent. Exercise training could not overcome these additional biomechanical demands when descending stairs in the common step-over-step manner. To our knowledge, this is the first demonstration in older adults that exercise training can positively affect stepping biomechanics when walking down stairs.

TABLE 4 | Sagittal hip joint moments of older adults descending stairs of different dimensions before and after 16 weeks of exercise training.

	Exercise group (n = 8)		Control group (n = 7)		
Gait cycle events	**Pre**	**Post**	**Pre**	**Post**	***p*-value**
Standard stairs					
Initial foot contact (0%)	−0.23 ± 0.10	−0.16 ± 0.09	−0.15 ± 0.07	−0.17 ± 0.06	0.09
Single-leg stance	−0.13 ± 0.16	0.01 ± 0.21*	−0.20 ± 0.15	0.12 ± 0.17*	0.003
Double support	−0.18 ± 0.19	0.10 ± 0.21*	−0.19 ± 0.25	0.19 ± 0.20*	0.001
Foot off	−0.23 ± 0.11	−0.11 ± 0.18*	−0.25 ± 0.11	−0.14 ± 0.12*	0.01
Final foot contact (100%)	−0.11 ± 0.08	−0.09 ± 0.08	−0.11 ± 0.09	−0.06 ± 0.06	0.58
Increased rise stairs					
Initial foot contact (0%)	−0.20 ± 0.14	−0.19 ± 0.13	−0.17 ± 0.18	−0.10 ± 0.06	0.57
Single-leg stance	0.23 ± 0.32†	0.03 ± 0.25	0.14 ± 0.49	−0.07 ± 0.31	0.97
Double support	0.38 ± 0.28†	0.29 ± 0.32	0.30 ± 0.31	0.31 ± 0.23	0.31
Foot off	−0.17 ± 0.07†	−0.18 ± 0.10	−0.15 ± 0.08	−0.19 ± 0.11	0.36
Final foot contact (100%)	−0.06 ± 0.09	−0.08 ± 0.06	−0.05 ± 0.10	−0.08 ± 0.08	0.95

*Values are mean ± SD. Significant difference: training *, step dimension* $^{\dagger}p < 0.05$.

Effects of Exercise Training

Exercise training enabled older adults to use their ankle plantarflexors more and their knee extensors less, in standard rise stair descent. Age-related deteriorations in plantarflexor biomechanics exceed those of other muscle groups (Lark et al., 2003; Silder et al., 2008; Reeves et al., 2009), yet our training intervention increased maximum eccentric ankle joint moment (normalized to body mass) and dorsiflexion angle by 35 and 11%, respectively, when assessed by isokinetic dynamometry. During stair descent the absolute ankle joint moment increased after training, peaking with the left, load-bearing foot in single-leg support (at maximum dorsiflexion angle), when stepping down with the right foot; and in the trailing left foot after double support, to negotiate the load-bearing step. The latter phase corresponded to the second ankle, and then knee joint moment peak, and involved a large sagittal plane separation between the CoM and CoP indicating anterior lean (CoM in front of CoP), and subsequently posterior lean (CoM behind CoP) from the left foot off.

After exercise training, there was greater posterior leaning (i.e., the CoM distributed further behind the application of CoP) to control lowering of body mass in single-support when stepping down (**Figure 3A**). This may reflect a safer balance control strategy through positioning of CoM further behind the CoP after exercise training, reducing the risk of falling forward during stair descent. The compromise, however, is that this strategy requires higher joint moments with the ground reaction force being applied more posteriorly, which would consequently generate a larger external moment arm, but this was enabled through the increased joint moment capability provided by exercise training. Increased hip joint flexion moment in this early-swing phase is unlikely to have contributed to these differences in balance control (Simonsen et al., 2012). Exercise training, particularly leg-press and calf-press contractions, strengthened ankle and hip musculature, and enabled participants to tolerate the development of higher joint moments and adopt a more posterior CoM displacement. This joint moment redistribution was accommodated by reduced knee joint moment in a phase of high demand (swinging the left leg to step down), as the maximum knee capabilities measured by dynamometry also improved.

We combined resistance exercise with ankle stretching for the following two reasons. Firstly, limitation of mobility of the ankle and knee joints is prevalent in aging, and these in turn, may impair dynamic stability when stepping down in stair descent (Bosse et al., 2012). Secondly, unlike the knee joint (Andriacchi et al., 1980; Mian et al., 2007), the ankle approaches it's maximum dorsiflexion joint range (∼20–30°) (Protopapadaki et al., 2007; Reeves et al., 2008a), and moment limits (∼75%) when descending stairs, and so has less reserve capacity than the knee joint. Thirdly, in combination these present high falls risk, due to limited ankle motion predisposing to a "controlled fall," and limited ankle force development comprising the individual's ability to respond to unexpected perturbations when stepping down.

Training-induced strength changes may have increased the reliance upon ankle joint moment to sustain single-leg support in stair descent. Redistribution of lower-limb joint moments occurred at a phase of high demand in stair descent. In particular, the capability for the ankle joint to operate beyond maximum capacity at extreme dorsiflexion joint range. The maximum eccentric joint moment was greater after exercise training by 30 to 43% at the knee (angular velocity-specific), and by 35% at the ankle (**Table 1**). When joint moments in stair descent were normalized to maximum eccentric moments quantified by dynamometry, the knee exceeded (103%) and ankle neared (89%) maximum strength capabilities. After exercise training, the knee operated at lower (67%), and the ankle at higher (139%) proportion of maximum (**Tables 2**, **3**). This may seem paradoxical, however, during standard stair descent the gastrocnemius would have been contracting in a bi-articular manner, acting across the ankle and knee joints. Whilst during isokinetic dynamometry testing, the gastrocnemius would have been contracting in an isolated, uni-articular manner,

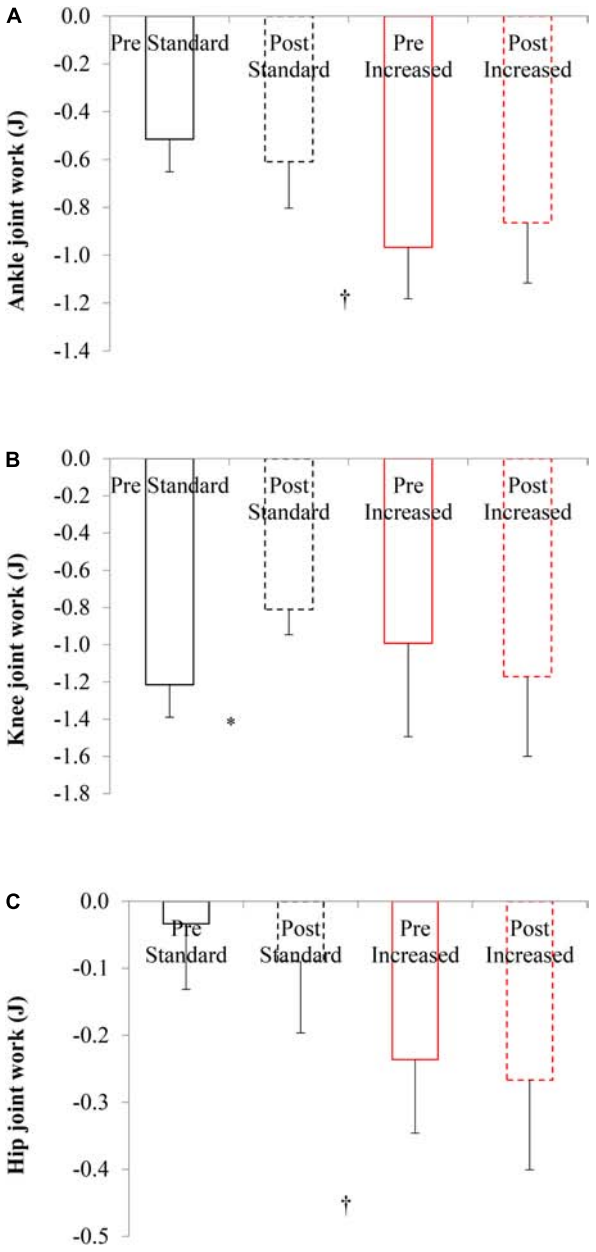

FIGURE 2 | Joint work of older adults descending stairs of different dimensions before and after 16 weeks of exercise training for the ankle **(A)**, knee **(B)**, and hip **(C)** joints. Values are mean ± SD (n = 8); negative = eccentric work. Significant difference: training *, step dimension $^{\dagger}p < 0.05$.

and thus producing lower summated force. Similarly, Samuel et al. (2011) reported knee extensor moments in stair descent exceeding maximum isometric moments by 120% for adults aged over 60 years.

The excessive task demands of stair descent leave minimal reserve, with which older adults can capitalize on in unexpected situations. Mian et al. (2007) combined aerobic and resistance exercise training for older adults over 12 months, without demonstrating change in gait speed and kinematics in stair descent. We progressed on this intervention by incorporating stretching and rate of torque development exercises, specifically for the ankle joint; that which is the limiting-factor at the most demanding point of stair descent (i.e., single-leg support). Additionally, in quantifying gait kinetics and movement strategies we have demonstrated that 16 weeks of exercise training can lead to older adults descending stairs with redistributed joint moments and altered postural stability.

During descent, the joint work was negative, as energy was absorbed. Before exercise training the knee joint performed the most negative work (−1.22 J), when compared to the ankle (−0.52 J), and hip (−0.03 J) joints. However, negative work by the knee joint significantly reduced after exercise training (−0.81 J), whilst remaining similar at the ankle and hip joints (**Figure 2**). This may signify that less energy absorption (in eccentric quadriceps contraction) was required in decelerating the knee joint when stepping downward. Theoretically this would require the older adults to generate a narrower impulse (i.e., applying higher torque more rapidly to adequately decelerate body mass). It appears after exercise training the lower-limb joints share energy absorption through the swing phase (kinetic energy production) to stepping down (dissipation). This has relevance for stair safety and potentially injury, as stair descent requires the knee joint to absorb 3.8 times more maximum power, than level walking (Riener et al., 2002). The present positive results of exercise training on stepping biomechanics in older people executing the standard rise descent task are particularly relevant for reducing the risk for stair falls, as most of the private and public staircases encountered daily are regulated, with a rise around the 170 mm value examined here. Interventions to improve stair safety in older people should therefore consider improving not only the environment (Jacobs, 2016), but also the individual's functional capacities.

Effects of Increasing Riser Height

The second part of this study involved investigating whether descending stairs of increased riser height would impact on the biomechanical strategies adopted by older adults, and subsequently, whether 16 weeks of exercise training could further affect their biomechanics. Our experimental staircase was configured to closely replicate the ranges of stair riser height negotiated in daily life, that is, standard stairs (170 mm) and increased rise stairs (255 mm). Standard stair descent provided an experimental benchmark, with which to compare the biomechanical demands to increased rise stair descent. Increased rise stair descent represented a much higher task demand that may be encountered in certain circumstances, for example, in steps on public transport (Institute for Transportation, and Development Policy [ITDP], 2018), pre-1950s residential dwellings and unregulated staircases (HM Government, 2013). Descending stairs with a rise of 255 mm is demanding for the young, particularly at the ankle and knee joints (Spanjaard et al., 2008). Adopting the common step-over-step strategy in the present study, a 255 mm rise required the older participants to increase step length (14.3%), reduce stride frequency (−17.7%) and prolong single-leg support (24.6%) in lowering greater

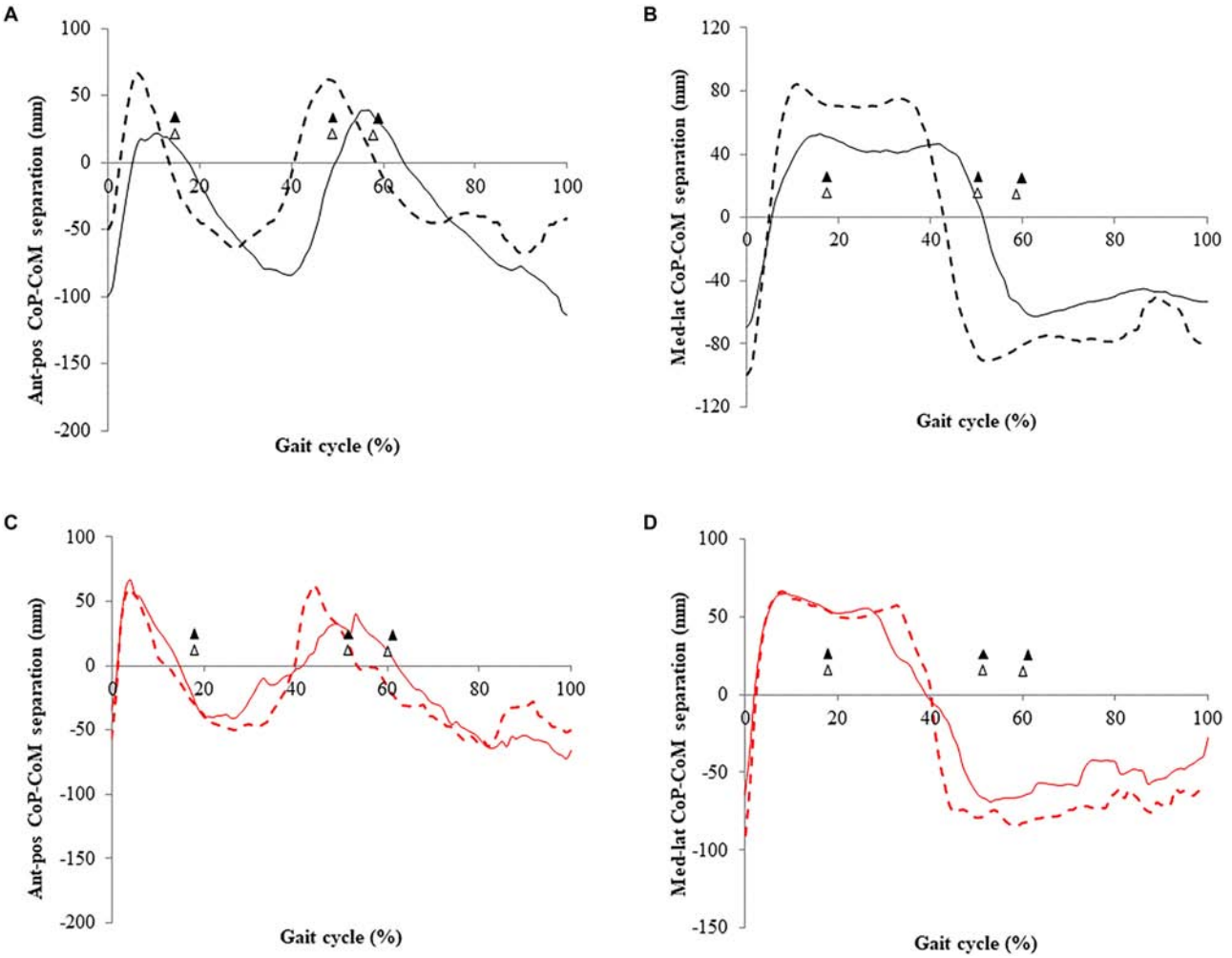

FIGURE 3 | Center of pressure (CoP) and centre of mass (CoM) separation of older adults descending stairs of different dimensions before and after 16 weeks of exercise training. **(A)** Anterior-posterior separation (standard stairs); **(B)** medial-lateral separation (standard stairs); and **(C)** anterior-posterior separation (increased rise stairs); **(D)** medial-lateral separation (increased rise stairs). Gait cycle events (▲ standard, △ increased) refer to initial foot contact (0%), single-leg stance, double support, foot off, and final foot contact with the above step (100%); data correspond to the left limb. Values are mean (n = 8).

vertical distance to the below step. As in the young (Riener et al., 2002), descending stairs of increased rise involved higher joint moments at the ankle, knee and hip in the old. The point of highest demand was in single-leg support, which involved peak dorsiflexion and high knee extension moments. From left foot contact in forward continuance, the knee flexion joint moment was greater on increased rise stairs until the right swing phase to the step below. This coincided with lower knee extension moment, when compared to standard rise stairs.

Whereas standard stair descent was most demanding on the knee joint, by increasing riser height the greatest demand was placed on the ankle joint. According to dynamometry-normalized joint moments, at the point of highest demand the ankle and knee joints were operating at 89 and 103% of maximum capacity in stair descent, respectively. Conversely, descending increased rise stairs required the ankle joint to work at 175%, and the knee joint 76% of maximum moment capacity at the point of highest demand. As discussed above, the ankle operating beyond maximal capacities after training may be attributed to a gastrocnemius bi-articular action for (dynamic) stair descent, and uni-articular action for (static) isokinetic dynamometry testing.

No falls or events of postural instability occurred in stair descent in our cohort, indicating that these community-dwelling older adults were capable of coping with excessive demand at the ankle joint. However, for adults with functional limitations at the ankle it would be extremely difficult to safely descend stairs of increased rise unaided. Where environmental aids are not present, older adults can adopt non-cyclical gait strategies (e.g., step-by-step or side-stepping) to control gait speed, without prolonging single-leg support in stair descent. For example, side-stepping can be used to reduce ankle plantarflexor and hip extensor demand, without enlarging the lead limb joint moments upon step contact (King et al., 2018). These compensatory strategies, however, involve the preferential use of a single lead

TABLE 5 | Centre of pressure (CoP) and centre of mass (CoM) separation peaks of older adults when descending stairs of different dimensions before and after 16 weeks of exercise training.

	Exercise group (n = 8)		Control group (n = 7)		
Anterior-posterior	**Pre**	**Post**	**Pre**	**Post**	***p*-value**
Standard stairs					
CoP-CoM separation (mm)					
Total excursion	183.6 ± 32.7	169.0 ± 37.8	166.6 ± 23.5	167.2 ± 38.0	0.42
Maximum (positive): CoM behind CoP	58.2 ± 20.8	79.2 ± 33.5*	58.9 ± 25.0	70.7 ± 25.1	0.011
Minimum (negative): CoM in-front of CoP	−125.4 ± 17.6	−89.8 ± 21.0*	−108.0 ± 11.3	−96.2 ± 22.0	0.018
Medial-lateral					
Total excursion	143.6 ± 36.7	192.4 ± 37.1*	147.6 ± 16.7	163.4 ± 24.4	0.024
Maximum (positive): Right medial inclination	64.7 ± 17.6	85.4 ± 14.8**	63.9 ± 7.2	69.8 ± 12.4	0.017
Minimum (negative): Left medial inclination	−78.9 ± 26.6	−107.0 ± 23.2**	−83.3 ± 15.1	−93.7 ± 16.2	0.004
Increased rise stairs					
Total excursion	187.2 ± 20.0	174.1 ± 10.9	177.2 ± 19.0	177.8 ± 11.0	0.25
Maximum (positive): CoM behind CoP	94.5 ± 12.5	94.9 ± 13.8	94.8 ± 21.8	86.2 ± 11.9	0.10
Minimum (negative): CoM in front of CoP	−92.7 ± 32.0	−79.1 ± 16.2	−82.5 ± 15.0	−92.1 ± 14.8	0.13
Medial-lateral					
Total excursion	161.2 ± 19.8	172.0 ± 31.1	165.0 ± 42.7	160.2 ± 32.7	0.26
Maximum (positive): Right medial inclination	71.2 ± 13.1	75.5 ± 26.0	68.1 ± 25.0	69.2 ± 21.1	0.80
Minimum (negative): Left medial inclination	−90.0 ± 13.2	−96.5 ± 7.9	−97.0 ± 18.5	−91.0 ± 15.8	0.12

*Values are mean ± SD (n = 8). Significant difference: training *$p < 0.05$.*

TABLE 6 | Velocity and acceleration of older adult's center of mass (CoM) when descending stairs of different dimensions before and after 16 weeks of exercise training.

	Exercise group (n = 8)		Control group (n = 7)		
	Pre	**Post**	**Pre**	**Post**	***p*-value**
Standard stairs					
CoM peak velocity (m/s)	−0.43 ± 0.07	−0.45 ± 0.04	−0.49 ± 0.08	−0.50 ± 0.05	0.93
CoM peak accel. (m/s^2) descent phase	−2.2 ± 0.6	−2.4 ± 0.4	−2.3 ± 0.7	−2.7 ± 0.7	0.58
CoM peak accel. (m/s^2) landing phase	1.4 ± 0.5	1.7 ± 0.6	1.9 ± 0.6	1.8 ± 0.4	0.057
Peak foot velocity (m/s)	−1.39 ± 0.11	−1.36 ± 0.11	−1.54 ± 0.19	−1.61 ± 0.16	0.09
Increased rise stairs					
CoM peak velocity (m/s)	−0.58 ± 0.09	−0.55 ± 0.08	−0.64 ± 0.09	−0.66 ± 0.14	0.17
CoM peak accel. (m/s^2) descent phase	−3.3 ± 0.7	−3.3 ± 0.8	−3.7 ± 1.1	−4.4 ± 1.1*	0.04
CoM peak accel. (m/s^2) landing phase	2.1 ± 0.4	2.1 ± 0.2	2.3 ± 0.6	2.5 ± 0.7	0.34
Peak foot velocity (m/s)	−1.64 ± 0.21	−1.51 ± 0.26	−1.81 ± 0.19	−1.76 ± 0.25	0.54

*Values are mean ± SD (n = 8). Significant difference: training *$p < 0.05$.*

limb, which may be unsuitable to recover bilateral function in individuals with unilateral muscle (Metcalfe et al., 2013).

For standard stairs, exercise training was effective in alleviating the mechanical knee joint work in descent. From an interventional perspective, mechanical joint work was more heavily influenced by stair dimension, than exercise training. Increased rise stairs required greater negative work at the ankle (−0.97 J) and hip (−0.24 J) joints in descent, when compared to standard stairs (**Figure 2**). Riener et al. (2002) found similar involvement for increased joint power maximums at the ankle (67.3%) and hip (24.3%) when increasing stair inclination during descent. Stair descent involves large dorsiflexion joint range and support moments; if an individual lacks ankle joint range and/or functional strength when descending increased rise stairs, the lowering of mass to the below step will be greatly compromised. For this reason, our older cohort underwent lower limb resistance exercise, with rate of torque development and stretching specifically for the ankle plantarflexors, with which to enhance maximum dorsiflexion motion and support moments in the lowering phase. Thus, an important step in developing future exercise-based interventions would be to prioritize the ankle joint, particularly targeting plantarflexor muscular capacities for older adults in an ecologically valid environment.

The altered gait pattern required to safely descend increased rise stairs not only imposed greater functional demand, but also challenged movement control. Descending stairs of increased rise resulted in higher CoM and lead foot velocities, and CoM acceleration toward the ground, when lowering to the below step. The descriptive statistics indicate greater variability in CoM

motion (see **Table 6**) when descending increased rise stairs. This is reasonable considering the greater vertical distance that must be overcome when stepping downward with increased rise steps. It is equally plausible that this added demand also contributed to the slightly faster CoM peak downward acceleration (from -3.7 m/s^2 to -4.4 m/s^2; d = 0.28) in the control group when descending increased rise stairs, after 16 weeks of no training. The speed at which CoM downward velocity is arrested on landing in stair descent is mediated by the ankle joint's capability to produce eccentric torque at high angular velocities (Buckley et al., 2013). With older adults already operating at near maximum ankle capacity for standard stairs, and in-excess of maximum for increased rise stairs, individuals would have to manipulate their movement strategy. This was characterized by a reduced CoP-CoM displacement in the frontal plane (i.e., medial-lateral direction), and larger CoP-CoM displacement in the sagittal plane (i.e., anterior-posterior) during increased rise stair descent.

Descending increased rise steps (255 mm) requires greater plantarflexor and dorsiflexor activity upon landing (Gerstle et al., 2018), which may reflect a challenge to medial-lateral stability. Increased maximal ankle capacities following exercise training may have contributed to assist in controlling medial-lateral CoM stability. Progressing forward into single-leg support with the right foot leaving the above, increased rise step, the CoM was positioned further behind the CoP of the left foot, indicating greater posterior lean in descent (**Figures 3A,C**). Thereafter, from the right swing phase into double support the CoM was positioned closer to the CoP, indicating reduced anterior lean. No previous study has reported functional demand and balance control at the ankle, knee, and hip joints during increased rise stair descent for healthy, older adults. Greater trunk posterior leaning when lowering body mass further on to the below step, and lesser anterior leaning, upon the step contact (and mass acceptance) can be seen as a safe strategy in preventing uncontrolled CoM acceleration.

Effects of Exercise Training at Increased Riser Height

Older adults adopt cautious movement strategies in stair descent to safely maintain lowering of mass, including prolonging the trail limb muscle co-contraction at the ankle and knee (Buckley et al., 2013) and reducing lead foot heel clearance (Kunzler et al., 2017). By increasing the riser height, and therefore task demand, our healthy older adults adopted alternative movement strategies to control safe stair descent. However, 16 weeks of exercise training was ineffective in enabling participants to further cope with the additional demands of increased rise stair descent. Our increased riser steps (255 mm), were only 15% higher than the maximum rise recommended for new private domestic stairs (220 mm) (HM Government, 2013). Community-dwelling older adults can be expected to negotiate similar riser heights in private and public buildings constructed prior to new national regulations.

The knee joint produced greater moment in single-leg support on increased rise stairs after exercise training. However, when normalized to dynamometry-assessed maximum eccentric moments, the exercise training had no effect on lower-limb joint moments in stair descent. Similarly, the amount of negative work performed by a lower-limb joint was constrained by stair rise, and not influenced by exercise training. Training improvements in ankle torque production may have been sufficient to support the contralateral limb, as body mass was lowered to the step below, but for increased rise stairs, it appears ankle adaptations could not overcome the additional demand of lowering greater vertical distance. In this case, greater knee extension moments when descending increased rise stairs following training may have compensated, particularly to counter large knee flexion angles, which exceed those of standard rise stairs (Protopapadaki et al., 2007).

In the present study, participants descended each stair step-over-step, at a self-selected pace. Therefore, by necessity the descent tasks were different between stair configurations, but theoretically identical before and after training. However, as stride frequency lowered for increased rise stair descent after training, the same motor task was not being performed. Whether this reflects a more stable and controlled gait is unclear as movement control remained unchanged after exercise. Unsurprisingly, exercise training did not affect balance control (i.e., CoP-CoM separation) when descending increased rise stairs. The greater downward distance to the below step required not only an altered movement strategy to control CoM velocity, but also the ankle joint to operate beyond functional capacity. The increased rise was too high to "allow" any adaptation after exercise training. This is also supported by the downward CoM velocity and acceleration, which remained unchanged. The improvements in maximum functional capabilities at the ankle, knee and hip were nullified by the requirement to further control CoM acceleration and to generate sufficient external joint moments with which to alter postural strategies (i.e., CoP-CoM separation). Our older cohort was instructed to descend stairs using the step-over-step gait pattern, and therefore participants could not adapt their strategy to reduce the biomechanical demand. This further indicates that the increased rise stairs posed maximal demand, for which exercise training could not overcome, as opposed to older adults having to adopt differential gait strategies (e.g., side-stepping) to cope with increase rise descent.

CONCLUSION

This study demonstrated that combined, lower-limb resistance and stretching exercise training can confer functional improvements in older adults when descending a staircase with a standard 170 mm riser height. Specifically, enabling our older cohort to redistribute lower-limb joint moments and adopt movement strategies to cope with the task demands of stair descent, including a safer balance control strategy. However, exercise training could not overcome the extra biomechanical demands imposed

by increasing riser height by 50%. The post-exercise improvements at 16 weeks in the standard riser descent task highlights the relevance of incorporating appropriate exercise training in interventions aiming at improving daily stair safety in older people.

ETHICS STATEMENT

This study was carried out in accordance with the recommendations of the Ethics and Governance guidelines of the Manchester Metropolitan University, with written informed consent from all participants. All participants provided written informed consent in accordance with the Declaration of Helsinki. The protocol was approved by the ethics committee of the Manchester Metropolitan University, United Kingdom. We can confirm no vulnerable populations were involved as participants in the study.

AUTHOR CONTRIBUTIONS

NR, CM, JB, VB, DJ, and MR contributed to the conception and design of the study. NR performed the data collection. JG performed the subsequent data and statistical analyses. JG, NR, CM, JB, VB, and MR interpreted the results of the research. JG wrote the first draft of the manuscript. NR, CM, JB, VB, and MR reviewed the manuscript, as JG revised the subsequent manuscript versions. All authors contributed to the final manuscript revision, and read and approved the submitted version.

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The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Comparison of Muscle Function, Bone Mineral Density and Body Composition of Early Starting and Later Starting Older Masters Athletes

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Masters endurance runners can epitomize healthy aging; being reflective of the physiological processes of aging without the compounded effects of inactivity. The primary aim of the present study was to determine, using cross-sectional data, whether individuals taking up training after the age of 50 years can achieve the same level of athletic performance and musculoskeletal characteristics in their older age as those who trained all of their adult lives. A total of 150 master endurance runners [age 68 (5) years; 111 male, 39 female] were divided into early starters (training all of their adulthood) and late starters (started training after age 50 years). A comparative non-athletic group of 59 healthy older adults [age 73 (4) years; 30 female, 29 male] were additionally included for analysis. Training intensity, age-graded performance (AGP) and musculoskeletal assessments were performed. Results showed that there was no difference between athlete groups for training intensity or age-graded performance, despite the 30-year difference in training history. Body fat percentage and leg lean mass did not differ between athlete groups, but were 17% lower and 12% greater, respectively, in athlete groups compared with controls. Power normalized to body mass did not differ between any groups. Spine BMD was lower in late starters than controls, while early starters did not differ from late starters or controls. Hip BMD did not differ between any of the groups. These findings show that the Masters athletes we studied that started intense endurance running after the age of 50 years had lower body fat and higher leg lean mass compared to non-athletes. Body composition and athletic performance of the late starters was very similar to those who trained all of their adult lives.

Keywords: masters athletes, body composition, bone mineral density, starting age, endurance running

INTRODUCTION

There are growing numbers of older people training and competing as Masters runners in mass-participation endurance events, such as 5, 10 km, Half Marathon or full Marathons (Stevinson and Hickson, 2014; Lepers and Stapley, 2016). At the highest competitive level, Masters runners train 4–7 times per week at high intensity (Piasecki J. et al., 2018). Their overall lifestyle includes more physical activity than the general population, as they accumulate around 3-fold more low impacts as a result of voluntary movement during general activities, 20-fold more medium impacts and 200-fold more high impacts (measured in units of gravity, *g*) compared with typical older adults from the general population (Deere et al., 2016; Hannam et al., 2016b).

Intense training sustained through middle and into older age helps to maintain low body fat percentage (Piasecki et al., 2019) and, possibly, greater lean mass and skeletal muscle strength into old age. However, the available evidence regarding muscle mass and function of older Masters runners is conflicting. A recent meta-analysis highlighted research gaps due to most studies having low participant numbers with very few female athletes, limited outcome assessments and unclear demographics (Mckendry et al., 2018). For example, two studies (Power et al., 2012; Stenroth et al., 2016) showed higher maximal force or power in Masters endurance runners compared with non-athletic old, but several others showed no difference between Masters endurance runners and controls (Aagaard et al., 2007; Michaelis et al., 2008; Mikkelsen et al., 2013; Couppe et al., 2014; Piasecki et al., 2016; Piasecki J. et al., 2018) or even lower (Sipilä and Suominen, 1991; Sanada et al., 2009) maximal force or power in Masters endurance runners compared with non-athletic old. More recently, Fien et al. (2017) reported high levels of physical function, low body fat, and good health status of Masters athletes, but these characteristics declined with advancing age and because no comparison was made with non-athletic old it is not possible to know if these characteristics were any better than typical healthy old. Differences between studies in the athletic ranking of Masters athletes (e.g., highest achievements), age and years of training are possible explanations for the conflicting reports, but there is very little information available to show how the starting age influences physical performance, muscle mass, and body composition in later life.

In the general population, leg skeletal muscle mass declines progressively from age 30 years at a rate of approximately 8–10% per decade (Lynch et al., 1999; Janssen et al., 2000; Lee et al., 2004; Mitchell et al., 2012; Moore et al., 2014) and maximal force and power decline progressively at approximately 10–12% per decade (Michaelis et al., 2008; Dodds et al., 2014; Bagley et al., 2019). Therefore, by age 50 years non-athletic men and women have typically undergone two decades of progressive muscle declines. If regular endurance running preserves muscle structure and function into older age (Ringsberg et al., 2001; Landi et al., 2018; Shiroma et al., 2018), then life-long athletes should have avoided these age- and lifestyle-dependent declines and should have greater muscle mass and function than those taking up running after the age of 50 years. Although no research is available to support these possible benefits of longer-term training, there is evidence to suggest that starting age is an important factor. A previous study of Masters tennis players found that the upper limb (radius, ulna, and humerus) bone circumference and bone mineral density were greater in those who started training at an early age compared with those starting at an older age (Ireland et al., 2014). However, this study did not examine muscles or bones of the legs that would be exposed to "impacts" during running. More crucially, the positive influence of playing tennis from a young age for arm bones conflicts with other data suggesting that long-term endurance running does not give higher bone mineral density or may even be detrimental for spine, leg or hip bone mineral density (MacKelvie et al., 2000; Wilks et al., 2009a; Piasecki J. et al., 2018).

There is a gap in available knowledge of whether the age at which people take up competitive endurance running influences musculoskeletal structure and function as well as body composition in later life. These questions are important because regular exercise is recommended as the best way to combat age-related declines of the musculoskeletal system (Acsm, 1998; Kusy and Zielinski, 2015; Chief Medical Officer's Guidelines, 2018; Cruz-Jentoft et al., 2018) and the age at onset of exercise may affect health and performance-related indicators in later life. We therefore aimed to compare body composition, lean mass, maximal power, and hip and spine bone mineral density between Masters endurance runners competing all of their adult lives with those taking up competitive running after the age of 50 years. For reference, a non-athletic healthy older group was also included. It was hypothesized that athletic performance, lean mass, muscle power, and bone mineral density would be greater in trained compared with untrained adults, and greater in those with the longer history of athletic training compared to those starting athletic training after the age of 50 years.

MATERIALS AND METHODS

Study Design

The study was approved by the Regional Ethics Committee (North West England: 14/NW0275) as well the Local Ethics Committee of Manchester Metropolitan University. Written informed consent was obtained from all participants. Masters runners were recruited as part of the United Kingdom-based "VIBE" study (Deere et al., 2016; Hannam et al., 2016b). Male and female athletes were recruited from regional athletics meetings from across the United Kingdom. To be included, they must have been aged $\geq$59 years at the point of enrollment, had competed in endurance running in the past 12 months at a regional level and free from serious injury for over 2 years. They were invited to attend the research facility at Manchester Metropolitan University (United Kingdom) and were required to avoid training or competing for at least 48 h prior to attendance. The full cohort included 188 Masters Athletes, of which 33 were sprinters (events less than or equal to 400 m) and 150 were endurance runners (events greater than or equal to 800 m). Given the low number of sprinters (when separated to early starters, late starters, men, and women) and the known differences

in muscle mass and bone mineral density between sprinters and endurance runners (Gast et al., 2013; Kusy and Zielinski, 2015; Piasecki J. et al., 2018), the sprinters were excluded from further analysis for this particular study. Thus, a total of 150 Masters endurance runners were available for analysis. Mean age-graded performance (AGP) was determined by taking the athlete's highest ranked performance within the last 2 years and expressing it as a percentage of the world record for that age and distance. AGP ranged from 77–92% across the cohort, indicating a very high level of performance relative to age group records. For example, a 3 h and 30 min marathon for a 70-year-old man gives an age-graded performance of 80%, as described previously (Piasecki J. et al., 2018).

Data for control participants was taken from the MYOAGE study, a European multi-center study of healthy aging and the methodology has been described previously (McPhee et al., 2013). These participants were recruited by focused advertisement in newspapers, the third-generation university and the association of emeriti and universities, thus selecting cognitively active individuals living independently and without mobility impairments. An initial telephone interview was used to screen volunteers, which asked for self-reported number of exercise and social activity sessions per week over the past 3 years. Those who were sedentary defined as not involved in any regular activity sessions, as well as those involved in any form of regular, intense athletic or gym training were excluded from the control group. The controls did not complete accelerometry-based activity monitoring for use in this study. For the purpose of this study the 59 older participants recruited at the Manchester, United Kingdom, site and with complete data including hip and spine bone mineral density measurements were used.

Therefore, the total number of complete datasets available for analysis was 209 and all participants completed measurements at the same laboratory and using the same equipment. Of those, 140 were male and 69 were female. For this study, the athletes were divided into early starter (ES) and late starter (LS) athletes. These groups were based on answers from a questionnaire asking each athlete to self-report the number of hours spent training (0–1, 2–3, 4–7, over 7 per week) during different stages of their adult life (18–29, 30–49 and 50 years and over). Early starter athletes were defined as those that had taken part in competitive endurance running throughout their adult lives, reporting intense training and competition at ages 18–29, 30–49, and 50 years and over. Late starter athletes were defined as those that had taken up intense training and competition after the age of 50 years, with no previous competitive training history. See **Table 1** for participant characteristics.

Questionnaires

Participants provided demographic, general health, lifestyle, and physical activity information by questionnaire [described previously (Hannam et al., 2016b)]. Self-rated health was reported on a scale from very good through to very poor, along with details of any diseases or prescribed medications. Current and history of smoking was collected, including number of cigarettes smoked per normal day, age started smoking and the duration, if ever, of smoking. Typical alcohol consumption was recorded and the type of alcohol. Highest level of education was also recorded. Questionnaire response data has been provided in **Supplementary Table S1**.

Accelerometry

Accelerometry data was collected only in the athletes and has been described previously (Deere et al., 2016; Piasecki J. et al., 2018). Each athlete received a GCDC × 16–1c (Gulf Coast Data Concepts, Waveland, MS, United States), which was placed in a Velcro strap and worn tightly around the waist with the accelerometer device placed over their right hip. Each athlete wore a monitor for seven consecutive days, only removing it when showering, bathing, swimming, and sleeping. Each athlete completed a time sheet over the 7-day period to record when the monitor was worn and to indicate any reason why that day was not representative of their usual routine. Accelerometers were configured with standardized settings prior to participant use with a sampling frequency of 50 Hz, a deadband setting of 0.1 g (the threshold which must be exceeded before a recording is made) and a timeout setting of 10 s (meaning that a single sample every 10 s is taken even if the recording is $<$0.1 g) (Deere et al., 2016). Once the 7 day period of use was completed the participant returned the accelerometer to the research facility by post. The raw accelerometry data was uploaded to a secure shared drive and read into Stata 13 (StataCorp, College Station, TX, United States). A standardized cleaning and processing procedure was used and has been described in detail previously (Deere et al., 2016). In short, the *Y*-axis accelerations dataset was cleaned to remove any movement artifacts and any periods of nil data collection, most likely due to the participant not wearing the accelerometer. Activity data were normalized based on seven valid days of 14 h with $\geq$10 h recording time. *Y*-axis peaks were calculated based on accelerations that were higher than the previous and subsequent reading and recorded within 14 pre-specified g bands. These were condensed to three impact bands; low ($\geq$0.5 to $<$1.0 g), medium ($\geq$1.0 to $<$1.5 g) and higher ($\geq$1.5 g) impact. All *g* values represent *g* over and above 1 *g* from earth's gravitational force (Hannam et al., 2016a).

DXA Scans

Standing height was measured to the nearest millimeter and body mass was measured to the nearest 0.1 kg. Whole body, total hip and lumbar spine dual energy X-ray absorptiometry (DXA: Lunar Prodigy Advanced, GE Healthcare, encore version 10.50.086, London, United Kingdom) scans were performed while the participant lay supine wearing a light cotton t-shirt to reduce measurement errors due to clothing absorption. Body composition (fat mass and lean mass) was taken from results of total body scans and regional analysis of legs and arms. Bone mineral density (BMD, $g.cm^{-2}$) was taken from hip and spine scans. All measurements were recorded after manual adjustment of the regions of interest. Repeat total body and hip scans were performed in eight participants within 1 month of the first scan. Using these repeat scans, the short-term error for our laboratory was 2.0% for hip BMD, 0.9% for spine BMD and 0.01% for whole body lean mass.

TABLE 1 | Participant characteristics.

Variable	Group						ANCOVA	Pairwise comparisons			*Covariate*	
	(1) Early starter		(2) Late starter		(3) Controls			1 vs. 2	1 vs. 3	2 vs. 3	*Gender*	*Age*
Sex (n)	**M (48)**	**F (6)**	**M (63)**	**F (33)**	**M (29)**	**F (30)**						
Adulthood training years 95% CI	52.3 ± 6.0 50.7–53.9	47.6 ± 4.2 43.7–53.2	18.4 ± 5.1 17.1–19.7	19.4 ± 5.2 17.2–20.9	N/A	N/A	**<0.0005**	**<0.0005**	**<0.0005**	**<0.0005**	F(1,184) = 0.007, p = 0.934	F(1,184) = 286, p < 0.0005
Age (years)[a] 95% CI	71.3 ± 5.8 70.1–73.2	66.4 ± 3.0 62.9–71.6	68.8 ± 5.5 67.4–70.1	69.6 ± 5.1 67.7–71.2	74.1 ± 5.7 71.9–76.2	73.3 ± 4.5 71.6–74.9	**<0.0005**	0.095	**0.004**	**<0.0005**	F(2,242) = 15.8, p < 0.0005	n/a
Height (cm) 95% CI	171.2 ± 5.6 169.9–173.0	164.2 ± 4.5 158.4–167.0	174.0 ± 6.3 172.7–175.6	161.6 ± 6.9 159.5–164.2	172.0 ± 8.7 168.6–175.3	160.3 ± 5.1 158.4–162.3	0.411	–	–	–	F(1,242) = 0.142, p = 0.706	(1,242) = 0.455, p = 0.500
Body mass (kg) 95% CI	68.3 ± 8.7 67.2–72.4	55.4 ± 4.7 52.2–60.2	67.5 ± 6.8 66.8–70.3	56.1 ± 7.8 54.3–59.7	80.2 ± 16.2 74.1–86.4	63.1 ± 11.5 58.8–67.4	**<0.0005**	0.823	**<0.0005**	**<0.0005**	F(1,242) = 84.132, p < 0.0005	F(1,242) = 0.423, p = 0.516
BMI (kg/m^2) 95% CI	22.7 ± 4.3 22.1–24.5	20.5 ± 1.9 20.0–22.4	22.3 ± 1.9 22.1–23.1	21.6 ± 2.1 21.1–22.6	27.1 ± 4.7 25.3–28.8	24.5 ± 4.2 23.0–26.1	**<0.0005**	0.864	**<0.0005**	**<0.0005**	F(1,242) = 7.151, p = 0.008	F(1,242) = 0.080, p = 0.778
Accelerometry low impact (0.5–1 g) counts[b] 95% CI	33529 (21051–46725) 28539–40384	55066 (35520–64137) 19985–56368	44404 (32394–57262) 39101–50240	35637 (23960–52961) 32315–46670			0.089	0.089	–	–	–	F(1,175) = 1.48, p = 0.226
Accelerometry medium impact (1–1.5 g) counts[b] 95% CI	27565 (10700–50689) 20501–36133	29465 (23566–59147) 9973–42399	34901 (23648–49685) 28665–39081	29868 (20319–40853) 24249–40448			0.80	0.799	–	–	–	F(1,175) = 0.717, p = 0.398
Accelerometry (counts) high impact (>1.5 g) counts[b] 95% CI	172 (9–1214) 386–1217	89.6 (50–1572) −269.1–1511	221 (32–932) 549–3070	119 (10–745) −842–5272			0.35	0.352	–	–	–	F(1,175) = 0.035, p = 0.851
Age graded performance (%) 95% CI	74.5 ± 1.6 72.2–78.3	84.3 ± 2.2 83.3–92.6	77.8 ± 1.3 76.1–81.8	79.8 ± 1.9 76.5–83.8			0.29	0.294	–	–	–	F(1,174) = 4.137, p = 0.043

Data are mean ± SD. Age and sex were included as covariates, except where indicated by [a]where sex was the only covariate. [b]Data are presented as median (25th/75th) quartiles. 95% Confidence intervals (95%CI). Bold text is used to highlight statistically significant difference (p < 0.05).

Muscle Function

The investigators provided verbal instructions and a physical demonstration of the muscle function tests. Participants were allowed one practice immediately before the actual assessed trials, which acted as a specific warm up and also confirmed that the instructions were understood. In all cases, the muscle function tests were completed between 10 am and 3 pm.

Hand grip strength was measured using the Jamar dynamometer handle (Sammons Preston Inc., Bolingbrook, IL, United States) as previously described (Hannam et al., 2017). The width of the dynamometer was adjusted for each participant separately. Participants were instructed to stand upright with the arm fully extended along the body, maintaining approximately 5 cm gap between the wrist and the hip or upper leg (so that the hand was not rested against the body). Participants were instructed to squeeze against the handle as hard possible for 3 seconds. Grip strength was measured three times and recorded in kilograms to the nearest 0.1 kg. For the purpose of this study, the best of three attempts was included in further analysis.

A Leonardo Jump Mechanography Platform (Leonardo Software version 4.2: Novotiec Medical GmbH, Pforzheim, Germany) was used to assess lower limb muscle power during a countermovement vertical jump, as described previously (Hannam et al., 2017). Results for both absolute (W) and relative (W/kg) power were recorded. Briefly, a two-footed countermovement jump was performed starting with feet approximately 30 cm apart (slightly narrower than shoulder width) and standing upright on the force plates. Force was sampled at 800 Hz. Participants flexed at the knees before extending as forcefully as possible to take off for the jump. Jumps were performed with a trained research assistant in close proximity to intervene in case of a trip or fall. Each participant repeated the jump sequence three times, with approximately 60 seconds rest between efforts. The jump with the highest value for power was used for statistical analysis.

Statistical Analysis

Statistical analysis was performed using SPSSv21 (IBM, United States). Normality of distribution was assessed using the Shapiro–Wilk test, which showed that all data was normally distributed (presented as mean ± standard deviation) except for accelerometry data (presented as median (25th/75th) quartiles). Two-way ANOVAs (three group and two sex; all $p > 0.05$) showed no group × sex interactions for any of the variables so for this reason, together with the relatively small numbers of females in the ES group, data from both sexes were included within the same analysis. Univariate ANOVA (ANCOVA) analysis was used to identify differences between the three groups (ES, LS, and C) with age and sex added as covariates, to account for the different mean ages and proportions of males and females between groups. Where significant differences were found, Fisher's Least Significant Difference pairwise comparisons were performed. Differences between groups were considered statistically significant at $p < 0.05$.

As participants included in our study were initially recruited to address other primary research questions, sample sizes for our analyses were fixed by the data already available. Our power to determine small, medium and large effect sizes can be ascertained from the observed effect sizes for the different outcome variables based on the partial eta squared ($\eta^2 p$), as recommended by Lakens (2013) and O'Keefe (2007) for use with ANOVA analysis including covariates (age and sex). A small effect size was set at $\eta^2 p = 0.01$, a medium effect size was set at $\eta^2 p = 0.06$ and a large effect size was set at $\eta^2 p = 0.14$. The values provided in the Results section show that the study was sufficiently powered to detect even small differences between groups for the main outcome variables of body fat percentage, lean mass, spine BMD, and vertical jump power.

RESULTS

Participant Characteristics

Participant characteristics are shown in **Table 1** and other health and demographic information has been provided in **Supplementary Table S1**. The ES athletes had been training for 30 years longer than LS athletes ($p < 0.0005$). Pairwise comparison showed there was no significant difference between ES and LS for AGP ($p = 0.294$). Accelerometry collected over 7 days showed similar impacts in the low, medium, and high g bands between ES and LS groups ($p = 0.089$, 0.799, and 0.352, respectively, as shown in **Table 1**).

There was no significant difference between groups for height. However, age, body mass, and BMI were significantly different between groups (all $p < 0.0005$). Pairwise comparisons showed that C were older than ES and LS, with no difference between ES and LS groups. Body mass and BMI were similar for the ES and LS athletes, but significantly greater for C compared with ES and LS groups (both $p < 0.0005$). The main reason for greater body mass in C was the higher body fat percentage (**Figure 1B**).

Muscle Function and Body Composition

Total body fat percentage differed significantly between groups ($p < 0.0005$; $\eta^2 p = 0.380$) due to approximately 17% lower values for both athlete groups than C ($p < 0.01$; **Figure 1A**). Leg lean mass differed significantly between groups ($p < 0.0005$; $\eta^2 p = 0.098$; **Figure 1B**), being approximately 12% greater in both athlete groups than C ($p < 0.003$). Appendicular lean mass was also different between groups ($p = 0.003$; $\eta^2 p = 0.062$) and although values were similar between athlete groups, the LS had a significantly greater lean mass than C ($p = 0.001$). Total body lean mass did not differ significantly between groups ($\eta^2 p = 0.028$; **Table 2**).

Spine BMD ($p = 0.004$; $\eta^2 p = 0.053$), BMC ($p = 0.001$; $\eta^2 p = 0.064$), and Area ($p = 0.014$; $\eta^2 p = 0.041$) were significantly different between groups. There were no differences for these measurements between athlete groups, but LS had lower BMD ($p = 0.001$; $\eta^2 p = 0.053$), BMC ($p < 0.0005$; $\eta^2 p = 0.063$), and Area ($p = 0.004$; $\eta^2 p = 0.041$) compared to C. There was no significant difference in Hip BMD ($\eta^2 p = 0.024$), BMC ($\eta^2 p = 0.020$) or Area ($\eta^2 p = 0.005$) between any of the groups (**Table 2**).

Maximal grip strength ($\eta^2 p = 0.020$) and vertical jump power relative to body mass ($\eta^2 p = 0.005$) did not differ

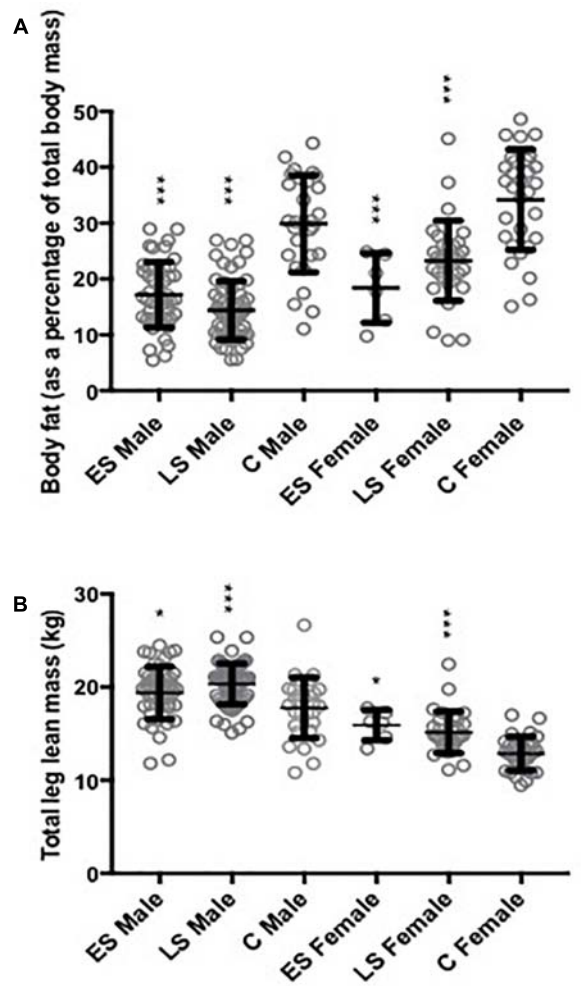

FIGURE 1 | Body fat percentage and leg lean mass. Data shown as mean ± SD and individual data points also displayed as open circles. **(A)** Body fat expressed as a percentage of total body mass, significance assessed when adjusting for Gender [$F(1,242) = 44.2$, $p < 0.01$] and age [$F(1,242) = 2.07$, $p = 0.15$]. **(B)** Leg lean mass significance assessed when adjusting for Gender [$F(1,242) = 179$, $p < 0.01$] and age [$F(1,242) = 8.40$, $p < 0.01$]. ES, Early Starter; LS, Late Starter; C, Controls. *** Significantly different to controls at $p < 0.01$ and * at $p < 0.05$.

significantly between groups (**Table 2**). However, vertical jump power in absolute values was different between groups ($p = 0.020$; $\eta^2 p = 0.017$); values were similar between athlete groups, but controls exhibited a greater power than LS ($p = 0.006$).

DISCUSSION

The novel contribution of the present study is the focus on the starting age of Masters endurance runners in relation to later life performance and musculoskeletal health indicators. This is supported by the relatively large sample size, depth of physiological profiling and, for the Masters athletes, objective characterization of habitual physical activities. Our findings demonstrate that the men and women of the LS group with no previous history of intense training or competition before the age of 50 years, had by the age of 70 years very similar training intensity, athletic performance, body fat percentage and leg lean mass to athletes of the ES group whom had accumulated 30 extra years of training and competition (**Table 2** and **Figure 1**). Both athlete groups had lower body fat and greater leg lean mass than healthy non-athletic controls, but spine BMD was lower in LS than in C. These findings suggest that starting regular, intense endurance running at, or after, the age of 50 years is not too late to compete at the highest level in Masters endurance running or to significantly delay accumulation of body fat and loss of leg lean mass in older age.

The physical activity levels of ES and LS equates to around 3-fold greater level of low impacts, 20-fold greater level of medium impacts and 200-fold greater level of high impacts per week compared with reference values that were previously reported for older adults from the general population (Hannam et al., 2016b). Past studies have demonstrated both a dose-response and an intensity-dependent response to regular exercise training, as more frequent and higher intensity training confer beneficial health- and performance- adaptations (Swain and Franklin, 2006; Bruce et al., 2008; McPhee et al., 2016). The high impacts and activity levels due to more vigorous intensity movements alongside the associated energy expenditure is the most likely reason why both athlete groups avoided the usual increase in adiposity with advancing older age (Elhakeem et al., 2017).

Appendicular and leg lean mass were similar between athlete groups, but both athlete groups had higher leg lean mass than C (**Table 2** and **Figure 1**). This is in line with two studies (Mikkelsen et al., 2013; Couppe et al., 2014) that reported greater muscle size of Masters endurance athletes than age-matched controls. These past studies (Mikkelsen et al., 2013; Couppe et al., 2014) included only 15 Masters athletes, compared with our much larger sample of 150 endurance runners with detailed activity tracking by accelerometry and confirmation of the high AGP. The prevailing view is that resistance exercise is needed to reduce risk of sarcopenia in old age (Cruz-Jentoft et al., 2018; Lee et al., 2018; Vlietstra et al., 2018). Our results suggest that long term intense endurance running is also effective, and that it does not matter if this activity is taken up after the age of 50 years. However, from the available data we are not able to identify an older age at which benefits of intense endurance running are diminished compared with those training for all of their adult lives.

One of the benefits of having larger muscles is the potential to develop greater muscle force and power. In our study, power measured by vertical jump was not different between athlete groups, but actually lower for LS compared with C. However, this difference between groups disappeared when normalized to total body mass (**Table 2**). Previous studies also reported similar vertical jump power in Masters endurance runners and age-matched non-athletic individuals (Michaelis et al., 2008). We did not determine the reasons why the larger muscles of endurance runners do not produce greater power, but it is likely related to the characteristic "slow" muscle fiber contractile properties of endurance athletes (Tanaka and Seals, 2008) which makes energy turnover more economical, but gives lower power as the product

TABLE 2 | Musculoskeletal characteristics.

Variable	Group						ANCOVA	Pairwise comparisons			*Covariate*	
	(1) Early starter		(2) Late starter		(3) Controls			1 vs. 2	1 vs. 3	2 vs. 3	*Gender*	*Age*
Sex (n)	**M (48)**	**F (6)**	**M (63)**	**F (33)**	**M (29)**	**F (30)**						
Total body lean mass (kg) 95% CI	53.3 ± 8.77 51.5–55.1	43.1 ± 9.48 40.6–45.5	54.8 ± 6.1 53.6–56.0	41.0 ± 9.4 39.1–42.8	52.3 ± 0.9 49.0–55.8	38.1 ± 4.3 36.4–39.5	0.215				F(1,242) = 280, p < 0.0005	F(1,243) = 6.29, p = 0.013
Total body fat mass (kg) 95% CI	11.9 ± 5.26 10.3–13.4	10.4 ± 4.02 6.14–14.6	9.92 ± 4.23 8.84–11.0	13.2 ± 5.06 11.4–15.1	24.6 ± 10.3 20.7–28.9	22.4 ± 9.00 19.0–25.7	**<0.0005**	0.494	**<0.0005**	**<0.0005**	F(1,203) = 0.477, p = 0.490	F(1,203) = 0.011, p = 0.917
Appendicular lean mass (kg) 95% CI	26.0 ± 0.5 25.0–27.0	19.7 ± 0.7 19.0–22.2	26.9 ± 0.5 26.2–28.1	19.0 ± 0.5 17.8–20.0	23.6 ± 0.8 22.0–25.4	16.5 ± 0.4 15.7–17.3	**0.003**	0.084	0.140	**0.001**	F(1,242) = 201, p < 0.005	F(1,242) = 10.9, p = 0.001
Spine BMD (g/cm^2) 95% CI	1.11 ± 0.02 1.09–1.18	0.93 ± 0.05 0.87–1.12	1.08 ± 0.16 1.06–1.13	0.88 ± 0.03 0.83–0.95	1.15 ± 0.03 1.08–1.21	0.95 ± 0.03 0.88–0.99	**0.004**	0.149	0.087	**0.001**	F(1,242) = 71.0, p < 0.005	F(1,242) = 0.77, p = 0.412
Spine BMC (g) 95% CI	271 ± 8.44 256–294	185 ± 18.6 140–303	249 ± 8.66 240–272	186 ± 1.16 173–203	300 ± 12.7 275–331	196 ± 11.3 167–216	**0.001**	0.091	0.073	**<0.0005**	F(1,242) = 60.2, p < 0.0005	F(1,242) = 0.070, p = 0.791
Spine area (cm^2) 95% CI	244 ± 4.32 238–253	196 ± 10.4 172–255	236 ± 3.44 231–244	202 ± 3.83 195–210	260 ± 6.16 249–277	204 ± 8.30 182–220	**0.014**	0.403	0.062	**0.004**	F(1,242) = 77.2, p < 0.0005	F(1,242) = 0.002, p = 0.966
Hip BMD (g/cm^2) 95% CI	1.05 ± 0.02 1.04–1.12	0.92 ± 0.06 0.85–1.03	1.02 ± 0.02 1.00–1.07	0.88 ± 0.02 0.85–0.93	1.05 ± 0.02 1.00–1.10	0.88 ± 0.02 0.81–0.92	0.094				F(1,236) = 58.7, p < 0.0005	F(1,236) = 8.57, p = 0.004
Hip BMC (g) 95% CI	39.7 ± 0.86 39.1–42.3	30.6 ± 1.96 28.5–34.4	37.2 ± 0.89 36.6–40.1	29.1 ± 0.72 28.1–30.8	39.0 ± 1.80 35.3–43.2	28.3 ± 0.94 25.8–29.9	0.135				F(1,236) = 95.5, p < 0.0005	F(1,236) = 0.372, p = 0.543
Hip area (cm^2) 95% CI	37.9 ± 0.53 37.1–38.9	33.4 ± 0.37 31.7–35.8	37.0 ± 0.70 36.1–38.6	33.3 ± 0.40 32.3–34.0	38.1 ± 063 37.3–39.7	32.3 ± 0.48 31.1–33.2	0.617				F(1,236) = 69.6, p < 0.0005	F(1,236) = 6.45, p = 0.014
Maximal grip strength (kg) 95% CI	36.8 ± 1.2 35.5–39.8	32.7 ± 3.4 29.1–37.5	37.3 ± 1.1 36.3–40.3	35.0 ± 1.9 31.9–39.1	38.2 ± 1.2 35.7–40.9	24.4 ± 0.9 22.3–26.1	0.142	–	–	–	F(1,236) = 32.3, p < 0.0005	F(1,236) = 6.20, p = 0.014
Vertical jump power (W) 95% CI	2054 ± 75.8 2016–2368	1359 ± 109 1169–1838	2014 ± 74.6 1967–2282	1420 ± 70.5 1314–1612	2191 ± 112.2 1997–2481	1463 ± 89.5 1243–1646	**0.022**	0.504	0.062	**0.006**	F(1,238) = 73.2, p < 0.0005	F(1,238) = 28.2, p < 0.0005
Relative vertical jump Power (W/kg) 95% CI	30.2 ± 1.1 29.1–33.5	24.5 ± 1.5 20.0–32.7	30.0 ± 1.1 28.8–33.0	25.3 ± 1.1 23.3–27.8	27.5 ± 1.0 25.8–29.9	23.0 ± 0.9 21.2–25.0	0.584	–	–	–	F(1,238) = 25.6, p < 0.0005	F(1,238) = 29.8, p < 0.0005

Data are presented as mean ± SD. Age and sex were included as covariates. Values highlighted in bold text identify significant differences between groups. BMD, Bone mineral density; BMC, Bone mineral content; %Female as displayed in ***Table 1****. 95% Confidence intervals (95%CI).*

of force x velocity of contractions (Michaelis et al., 2008). We did not measure knee extensor maximal force, but the available evidence is conflicting over the possibility that Master endurance runners have greater maximal force, mainly due to heterogeneity in study populations and several studies with low sample size (Mckendry et al., 2018).

The LS athletes had lower spine BMD, BMC, and Area than C, raising the possibility that starting regular intense endurance running after age 50 years may be detrimental to spine bone health. This may seem counter-intuitive, as it is proposed that regular exercise with high impacts can improve bone mineral density and bone strength (Ireland et al., 2011, 2013) as high muscular forces stimulate osteogenic responses (Frost, 1987a,b). However, this previous literature is primarily based on observations of long limb bones (Wilks et al., 2009a,b; Ireland et al., 2011, 2014). For example, previous studies showed that the limb bone circumference of Masters athletes (33–94 years old) was greater than that of age-matched sedentary controls (Wilks et al., 2009b). These beneficial effects may be limited to limb bones, younger ages or participation in sprint or power activities (Warden et al., 2007, 2014; Wilks et al., 2009b; Ireland et al., 2014). Previous research has also demonstrated an inverse association between the amount of low-impact physical activity and hip and spine BMD (Johansson et al., 2015; Hannam et al., 2016b; Piasecki J. et al., 2018). Although the explanation is lacking for why completing lots of low impact activity may be detrimental for bones, it is well known that bone responses depend on the type of physical activity being performed (Nichols et al., 2003; Velez et al., 2008; Wilks et al., 2009a; Piasecki J. et al., 2018; Pollock et al., 2018). For instance, sprinting is associated with greater hip, spine and tibial BMD compared to endurance running or non-athlete controls (Wilks et al., 2009a; Piasecki J. et al., 2018). Although not measured in the present study, Vitamin D and calcium intake are also associated with skeletal health in older age (Cashman, 2007). In particular, Vitamin D deficiency and low calcium intake can lead to low bone mineral density and they are used as supplements to combat osteoporosis (Grados et al., 2003a,b). Therefore, any interventions later in life to improve bone health may need to include nutritional supplementation, and also consider sprint or jumping activities in addition to regular prolonged endurance running.

Overall, our findings build upon available evidence that short-term exercise can improve some features of musculoskeletal health in middle- and older-age (McPhee et al., 2016) and have highlighted possible benefits of very long-term exercise (Velez et al., 2008; Wilks et al., 2009a,b; Nowak et al., 2010; Ireland et al., 2015; Mckendry et al., 2018), although these benefits may not be present for lumbar spine bones. The novel contributions of the present study describing the very long-term intense training and the focus on starting age are important because starting at a later age risks the possibility that irreversible age-related declines have already occurred and would limit adaptations that improve health and performance. For example, age-related reduction of maximal heart rate limits cardiac output and therefore peak aerobic capacity. Within the musculoskeletal system the skeletal muscle mass, strength and power decline from the fourth decade of life (Lynch et al., 1999; Janssen et al., 2000; Silva et al., 2010; Reid and Fielding, 2012; Moore et al., 2014; Pantoja et al., 2016; Fien et al., 2017; Bagley et al., 2019). The extent to which these changes are related to irreversible reductions of muscle fiber numbers (Lexell et al., 1988; McPhee et al., 2018) and motor units (Tomlinson and Irving, 1977; Piasecki M. et al., 2018; Piasecki et al., 2019) remains unknown. In this respect, the findings of the present study are encouraging. The implication based on observations made from the participants in our study, is that starting intense training in middle age, approximately 20 years after musculoskeletal declines are detectable in the general population, is not a disadvantage compared to training throughout adult life when it comes to maintaining leg muscle mass, preventing accumulation of excessive fat mass and for athletic performance in older age.

The findings of this study may inform practitioners when recommending physical activity for older adults aiming to reduce fat mass, gain leg lean mass or improve bone mineral density. The new information showing that long-term endurance training is associated with greater leg lean mass should be considered by policy makers as an alternative, or addition, to resistance exercise to combat sarcopenia. One note of caution, however, is that the lower bone mineral density of the spine in LS may increase the risk of late life bone injuries.

Study Limitations

The grouping of ES and LS has relied on self-reports and the cross-sectional study design prevents any causal relationships from being established. Our sample size of 209 provided sufficient power to detect even small differences in means between comparison groups, but we cannot rule out the possibility that there may be very small differences in outcomes between the ES and LS groups which we were unable to detect. There is also a risk of differences in data handling and screening of bone results (e.g., for spondylosis) when comparing the results of the Masters athletes with those of the controls, where data were collected approximately 5 years apart. However, care was taken to follow exactly the same protocols as far as possible and the assessments were made in the same laboratory using the same equipment. The values for body composition and lean mass of the controls are within the ranges previously published for non-athletic but otherwise healthy older adults from larger, multi-center studies (Bijlsma et al., 2013; Bauer et al., 2015; Coulson et al., 2017; Verlaan et al., 2017). Nevertheless, interpretation of outcome comparisons for the control group is limited because they did not complete the same physical activity assessments as the Masters athletes. The athletes were recruited based on their athletic performance over the previous 2 years. Within this time period there may have been fluctuations in activity levels due to injury or illness which were not captured within this study. Future studies could shorten the time period taken into account when recruiting the athletes or more carefully consider their time spent inactive. More detailed information about youth physical activity may also add further insights. A B-PAQ questionnaire (Weeks and Beck, 2008) may be used in future for this purpose. Studies of Masters athletes carry a possible bias because any individuals developing injury or disease may cease competing and would not be available

for recruitment. Masters athletes tend to be better educated, of higher socio-economic status and with fewer diseases than the general population (**Supplementary Table S1**), so the results of such studies may not be generalized to the wider population. Finally, we cannot rule out possible differences in energy intake or nutritional status, particularly of Vitamin D and calcium that influence musculoskeletal health, between athletes and controls because this was not measured in our study.

CONCLUSION

The Masters athletes within our sample taking up intense endurance running after the age of 50 years had lower body fat and higher leg lean mass than non-athletes by the age of 70 years and the values for body composition and athletic performance of the late starters were very similar to those of people whom had trained all of their adult lives.

ETHICS STATEMENT

The study was conducted in accordance with the Declaration of Helsinki and approved by the University Research Ethics Committee and the National Research Ethics Committee Northwest (14/NW0275). All participants provided written informed consent.

AUTHOR CONTRIBUTIONS

All authors contributed to the data collection, analyses, and wrote the manuscript. JP led the write up and directed the analysis of data.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphys.2019.01050/full#supplementary-material

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