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Nutrition, sarcopenia, and sarcopenic obesity

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Nutrition, sarcopenia, and sarcopenic obesity

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Editorial: Nutrition, sarcopenia, and sarcopenic obesity

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Editorial on the Research Topic
Nutrition, sarcopenia, and sarcopenic obesity

Sarcopenia, originally identified as loss of muscle mass, has recently been redefined as the independent and/or combined loss of muscle mass, strength, and muscle composition. The latter conceptual approach is associated with lipid deposition in muscle or myosteatosis leading to the pathologic condition of sarcopenia. Interestingly, sarcopenia primarily affects skeletal muscle and may indirectly affect cardiac or smooth muscle.

Sarcopenia is currently considered a public health crisis, estimated to affect 1–30% of community-dwelling individuals, 14–33% of institutionalized patients, nearly 25% of individuals over 65 years of age, and 60% of those over 80. The condition is reported in as many as 50% of patients with cancer, where it may vary with patient age and cancer type. Sarcopenia has been associated with various adverse health outcomes, including frailty, falls, fractures, functional decline, and increased risk of hospitalization and death. In addition to the progressive consequences of sarcopenia, its presence may impair the ability to effectively treat other comorbid conditions.

The development of sarcopenia appears to be multi-factorial and may be caused by multiple processes in addition to aging. These include nutritional deficiency or imbalance, lack of physical activity or sedentary behavior, hormonal imbalance, immunosenescence, mitochondrial dysfunction, inflammatory disorders, chronic diseases, and changes in the intestinal microbiome. Malnutrition (undernutrition, overnutrition, or nutrient deficiencies) contributing to sarcopenia has been proposed for multiple classes of proteins, amino acids, lipids, carbohydrates, and micronutrients including vitamins and minerals.

A particularly unique form of sarcopenia is the muscle loss and weakness that accompanies obesity, termed sarcopenic obesity (SO). This form of sarcopenia is also prevalent worldwide, particularly in older adults and in clinical settings. Individuals with SO are at higher risk of adverse outcomes, such as functional impairment and hospitalization.

The overall goal of this Research Topic of *Frontiers in Nutrition* was to more clearly elucidate the mechanisms contributing to the development of sarcopenia and sarcopenic obesity, to evaluate the impact of sarcopenia on other comorbid conditions, and to identify optimal therapeutic strategies and dietary interventions for the prevention, reversal, and/or management of sarcopenia.

There were 46 manuscripts submitted for this issue, of which 17 were published. The published manuscripts, as described below, focus mainly on the intersection of sarcopenia with other comorbidities including cancer, type 2 diabetes (T2D), cirrhosis, kidney stones, and severe abdominal trauma, among others. Several manuscripts examine the impact of dietary inflammatory indices on the development of sarcopenia, while others explore the importance of nutritional supplementation with and without exercise training.

In a cross-sectional study of 479 individuals with T2D, comprising 264 (55.1%) men and 215 (44.9%) women with a median age of 71 years, Shiroma et al. observed that 8.6% of patients presented with sarcopenia. To evaluate undernutrition status, they compared measurements of serum albumin, GNRI (Geriatric Nutrition Risk Index) based on height, weight, and serum albumin, CONUT (Controlling Nutrition Status) based on serum albumin, total peripheral blood lymphocyte count, and total cholesterol. Their study found the diagnostic power of GNRI to be superior to the measurement of albumin or CONUT for identifying sarcopenia, low skeletal muscle mass index, and low hand grip strength in patients with undernutrition and T2D. The authors discussed the possibility that sarcopenia in T2D may be caused by undernutrition due to therapeutic diets and/or anti-diabetic agents. They suggest the application of the simple GNRI tool to identify patients in need of nutritional support for the improvement of sarcopenia.

Yang Q. et al. reported on a clinical-based observational study of 217 patients (68.6% male), with a mean age of 67.3 ± 11.1 years, exhibiting diabetic foot ulcer (DFU), who were followed over 4 years at Chongqing Medical University. All participants underwent dual-energy X-ray absorptiometry (DXA) to determine body composition. A total of 33 (17.3%) of the 217 patients died. The 5-year survival for the entire group was 58.3%. Patients with sarcopenia had a reduced 5-year overall survival (OS) of 45.9%, compared to 85.4% OS for patients without sarcopenia. Age, sarcopenia, and serum creatinine were independent risk factors for all-cause mortality in these patients with DFUs. The authors suggest that active prevention of sarcopenia may increase survival in patients.

Several groups reported on the impact of sarcopenia in patients with cancer. Two studies focused on sarcopenia in patients with lung cancer and another two in patients with colorectal cancer (CRC). In a study of 126 newly diagnosed stage III and IV lung cancer patients, comprising 97 (77%) men and 29 (23%) women with a mean age of 64.8 ± 8.7 years, Wang et al. reported that the prevalence of sarcopenia and frailty was 25.4% and 32.5%, respectively. They reported the presence of low body mass index (BMI) to be 40% and low skeletal muscle mass measured by bioimpedance analysis (BIA) to be 38.1%. As sarcopenia is associated with chemotherapy toxicity and short survival, they suggest that nutritional intervention should be considered in patients with advanced cancer.

To more specifically evaluate the prognostic significance of sarcopenia in lung cancer patients undergoing targeted or immune therapy, Lyu et al. performed a retrospective analysis of 131 patients with advanced non-small cell lung cancer treated with first-line epidermal growth factor receptor tyrosine kinase inhibitors (EGFR-TKI) or immune checkpoint inhibitors (ICI). Among 35 (26.7%) patients with sarcopenia, median progression-free survival (PFS) and OS were 6.4 and 13 months, respectively, compared to those without sarcopenia, where PFS and OS were 15.1 and 26 months, respectively, and both $p < 0.001$. Importantly, sarcopenia and non-sarcopenia patients showed similar treatment-related toxicities; however, pretreatment sarcopenia predicted clinical outcomes in patients treated with EGFR-TKIs and ICIs.

Feng et al. evaluated the influence of sarcopenic obesity on treatment complications in patients undergoing radical resections for CRC. Conducting a retrospective cohort study at Beijing Friendship Hospital from January 2017 to May 2018, they evaluated 387 patients, with a median age of 64 years, of whom 63.6% were women, 36.4% were men, 247 had colon cancer, and 140 had rectal cancer. A total of 111 developed surgical complications, and 21 developed medical complications. Sarcopenia and obesity were determined by measuring visceral fat and skeletal muscle mass by CT scan at the L3-L4 intervertebral disc, with sarcopenic obesity defined as a high ratio of visceral fat area/skeletal muscle area. Among these patients, 198 (51.1%) were identified as having SO, while 189 (48.8%) were normal. Patients with SO were of higher age (66 years vs. 62 years), had more T2D, more blood loss at surgery, and showed more vascular invasion of the tumor as compared to their non-SO counterparts. This study showed that sarcopenic obesity is a high-risk factor for post-operative complications, particularly surgical complications. Based on these observations, the authors suggest that strengthening perioperative nutritional status may improve the short-term outcomes of surgery for CRC.

Sarcopenia generally exerts a negative outcome for patients with CRC. However, diagnosis of sarcopenia depends on instrument-based muscle mass measurements such as DXA, computed tomography (CT), or BIA, which are not always clinically available. Gao et al. evaluated the use of serum creatinine/cystatin C ratio (Cr/CysC) as a prognostic indicator in patients with CRC. Creatinine reflects muscle metabolism, whereas cystatin C, a non-ionic protein derived from all nucleated cells, is used to correct for glomerular filtration. This single-center retrospective study from the First Affiliated Hospital, Guangxi Medical University, Nanning, China, analyzed 975 patients (63% men, 37% women, median age 57.5 ± 13.1 years), comprising 494 (51.2%) patients with colon cancer and 476 (48.8%) with rectal cancer. They found 734 patients (75%) with a low Cr/CysC ratio (<106.25), indicative of low muscle mass, compared to 241 (25%) patients with a high Cr/CysC ratio, indicative of normal muscle mass. A low Cr/CysC ratio was an independent risk factor for PFS and OS in CRC patients. Patients with low Cr/CysC had lower 5-year OS of 52.5% vs. 68.9%. Kaplan–Meier analysis, according to disease stage, showed that patients with stage I-II CRC and low compared to high Cr/CysC ratio had a

decreased OS of 67.6% compared to 81.7%. Patients with stage III-IV CRC and low Cr/CysC had a median OS of 36.6% compared to 56.2% in patients with high Cr/CysC ratios. The authors suggest the use of Cr/CysC as a prognostic indicator of sarcopenia in patients with CRC.

Yang H. et al. performed a systematic review examining the impact of sarcopenia in patients with critical illnesses, such as sepsis, trauma, and surgery. The meta-analysis included 38 studies, totaling 6,891 critically ill patients, and observed a 51% pooled prevalence of low skeletal muscle mass (LSMM). Patients with LSMM compared to normal were likely to require mechanical ventilation, at 53.4% vs. 48.9%. Critically ill patients with LSMM compared to normal SMM had a significantly higher mortality risk, with an odds ratio (OR) of 2.35. Based on these studies, the authors indicate the need for early intervention, such as mobilizing patients and providing nutritional support for patients with LSMM.

Sarcopenia was further shown to interact with and impair multiple other conditions. In an analysis of National Health and Nutrition Examination Study (NHANES) data from the periods of 1999–2006 and 2011–2018, Tu et al. showed that among 7,829 participants with hypertension, 47.4% of whom were female and with an average age of 51.4 years, there were 1,352 patients with DXA-determined sarcopenia (17.3%). To assess the relation of dietary inflammatory factors on the development of sarcopenia, the diet inflammatory index (DII) was determined using 24-h dietary recall. Distributing patients from lowest to highest quartiles based on DII showed sarcopenia at 9.4%, 10.9%, 15.3%, and 19.3% of the respective groups. After fully adjusting for potential confounders, those in the highest quartile compared to the lowest quartile showed the highest risk for sarcopenia, with an OR of 2.43, $p < 0.001$. These studies showed that DII is significantly correlated with sarcopenia in patients with hypertension. It is noteworthy that there was no relation found between sex or anti-hypertensive medication as independent risk factors for sarcopenia. The authors stress the importance of the elderly maintaining nutrition with intake of energy, protein, fat, and other substances while maintaining an anti-inflammatory diet to prevent sarcopenia.

Further exploring the relationship between sarcopenia and comorbidities, Zhang et al. used the NHANES database to examine the association between sarcopenia and kidney stones in the adult population of the United States between 2011 and 2018. Among 9,432 participants, they identified 8,793 non-stone formers and 759 stone formers. Among all patients, after propensity matching, there was an OR of 2.365 for the association of kidney stone formers with sarcopenia. In patients of <40 years of age, the OR was 6.79, whereas for those over 40, the OR was 1.22. The authors conclude that sarcopenia is a potential risk factor for kidney stones in the US adult population.

Seeking to determine the relationship between skeletal muscle mass and nutritional status in patients hospitalized for abdominal trauma in a teaching hospital in China, Xi et al. retrospectively analyzed changes in skeletal muscle mass based on serial L3 CT scans in 103 patients, of whom 91 (88.3%) were male and 12 (11.6%) female, with a mean age of 43.74 ± 15.53 years. They observed a rapid decrease in skeletal muscle mass in week one post-trauma, with subsequent progression indicative of nutritional status and with loss of muscle mass being associated with poor prognosis. The authors indicate the need for further research to optimize recommendations for protein supplementation following acute trauma.

To gain a better understanding of the potential pathophysiologic processes mediating sarcopenia, Liu X. et al. compared plasma metabolomic and unsupervised principal component analyses in 20 patients with hepatitis B virus (HBV)-related cirrhosis and muscle loss to 20 patients with HBV-related cirrhosis without muscle loss and 20 healthy controls. A total of 70 differential metabolites were noted, of which 6 were upregulated and 64 downregulated in patients with muscle loss compared to those without muscle loss. The upregulated metabolites included ethylamine, (r)-3-hydroxybutyric acid, 3—hydroxymethylglutaric acid, 2-ketobutyric acid, 1,5-anhydroglucitol, and creatinine. These metabolites reflected disturbances in 25 pathways, most notably indicating an association of muscle mass loss in patients with cirrhosis, with disordered amino acid metabolism and central carbon metabolism in cancer. Based on these studies, the authors suggest the need to investigate the relationship between citrulline and muscle mass loss in patients with cirrhosis and the potential for the use of citrulline to support the arginine and protein synthesis pathway. Inosine 5′-monophosphate (IMP) was downregulated in patients with muscle loss. Since IMP can serve as a precursor for ATP and GTP, the authors suggest the need to evaluate IMP dietary supplementation to increase energy availability for protein synthesis.

Liu S. et al. provided a comprehensive scholarly review, including discussions of mechanisms and recommendations, for 13 nutritional supplements commonly employed in association with exercise and training to improve the quality of life of patients with sarcopenia. Protein and leucine supplements were recommended to support muscle protein synthesis, as were collagen peptides, although the latter were noted to be no more effective than other protein supplements. Creatine, a natural, non-protein amino acid, was noted to enhance response to exercise training among older adults. β-hydroxy β-methylbutyric acid was not recommended for dietary supplements. The authors suggest that further studies are still needed before recommendations can be made for supplementation with ω-3 fatty acids, inorganic nitrates, probiotics, minerals such as magnesium, selenium, and calcium, and polyphenols.

Ye et al. conducted a systematic review and meta-analysis to address whether dietary supplementation could prevent disuse muscle atrophy in subjects requiring periods of immobility. Examining muscle strength, cross-sectional muscle area, muscle fiber type, distribution, muscle volume, and peak aerobic capacity in 20 randomized control trials (RCTs) comprising 339 subjects, they conclude that dietary supplements with proteins, amino acids, and/or other nutrients, including creatine, ω-3 fatty acids, or β-hydroxy-β-methylbutyrate, extended no protective effect on muscle strength, cross-sectional muscle area, muscle fiber type, distribution, peak aerobic capacity, or muscle volume but did have a protective effect on lean mass. These results further support that nutritional supplementation alone does not prevent loss of muscle mass or strength but rather indicate the need for supplements to be coupled with exercise training to prevent disuse muscle atrophy.

Kim and Park evaluated the effect of ω-3 polyunsaturated fatty acids and dietary fish on the prevalence of low lean mass (LLM) and muscle mass in older women. Analyzing data on 1,620 men and 2,192 women over 65 years of age from the Korean National Health and Nutrition Survey, they found that consumption of EPA, DHA, and fish was negatively associated with the prevalence of LLM but positively associated with muscle mass in older Korean women, although not in men. The authors postulate that the beneficial effects of EPA, DHA, and fish may be associated with their anti-inflammatory effects.

Noting the association of dietary inflammatory potential with inflammatory cytokines, such as interleukin (IL)-1β, IL-4, IL-6, IL-10, tumor necrosis factor- α, and C- reactive protein, Xie et al. conducted a systematic meta-analysis of 24 studies covering 156,536 participants, in which they found that the consumption of diets with high dietary inflammatory index was associated with low skeletal muscle mass and increased risk of sarcopenia, with an OR of 1.53. They suggest the importance of diet strategies with increased intake of anti-inflammatory dietary components (fruits and vegetables) and decreased pro-inflammatory foods (sugar-sweetened beverages and processed meats) to prevent and treat sarcopenia.

To evaluate the potential therapeutic benefits of a complete nutrition drink fortified with anti-inflammatory EPA and branched-chain amino acids (leucine:isoleucine:valine), with the latter employed as muscle synthesis promoters, Khoonin et al. conducted a randomized, blinded placebo, controlled trial of nutrition supplementation and arm muscle exercise in 84 elderly men, with a mean age 65.15–67.86 years and with inadequate protein intake (<0.5 g/kg/day). After a 3-week intervention, they reported increased IL-10, reduced IL-6, and increased muscle mass and arm strength (measured by hand grip), particularly in patients with complete support; however, differences did not show statistical significance. These promising results suggest the need for randomized, controlled interventions of longer than 3 weeks' duration.

To evaluate a longer intervention, Vijayakumaran et al. conducted a nested, randomized, controlled 12-week pilot study of 16 older Malaysian women with possible sarcopenia measured by low grip strength and/or low 5-times sit-to-stand test, randomized to 3 times per week, undergoing high-intensity program resistance training (PRT) vs. PRT plus multi-nutritional WHEY protein supplementations (PRT + WP). There were six exercises, namely, squats, gluteal kickbacks, seated leg extensions, standing chest press, standing diagonal pull apart, and sectional rows, with exercise progression at 2, 4, and 8 weeks. They observed significant improvement in hand grip strength and stair ascent and overall improvement in patients with possible sarcopenia, but there were no significant differences between groups. The authors reported that the whey protein supplement was readily acceptable with limited side effects. The women indicated increased wellbeing from the intervention and wanted more nutritional information and structured, guided exercise programs. They further indicated their preference for a community-based program.

In conclusion, the research presented in this Research Topic of Frontiers in Nutrition has provided a profound understanding of sarcopenia's intricate relationship with various health conditions. From dietary inflammatory factors to the potential benefits of nutritional supplementation and exercise, this collection of research offers a comprehensive view of the field. These studies underscore the importance of early detection and intervention, emphasizing the role of nutrition in preventing and managing sarcopenia. As we look ahead, future research may explore personalized nutrition strategies, harnessing the potential of precision medicine to tailor optimal interventions for individuals. Additionally, investigating the role of emerging nutritional approaches and exploring innovative exercise regimens will be crucial for optimizing the care of patients with sarcopenia.

Author contributions

NB: Conceptualization, Project administration, Writing—original draft, Writing—review & editing. MY: Conceptualization, Project administration, Writing—review & editing. YC: Project administration, Writing—review & editing. CA: Project administration, Writing—review & editing. AS: Project administration, Writing—review & editing. WH: Project administration, Writing—review & editing. LK: Project administration, Writing—review & editing.

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A nutritional assessment tool, GNRI, predicts sarcopenia and its components in type 2 diabetes mellitus: A Japanese cross-sectional study

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Background: There are few reports evaluating the relationship between undernutrition and the risk of sarcopenia in type 2 diabetes mellitus (T2DM) patients.

Objective: We investigated whether undernutritional status assessed by the geriatric nutritional risk index (GNRI) and controlling nutritional status (CONUT) were associated with the diagnosis of sarcopenia.

Methods: This was a cross-sectional study of Japanese individuals with T2DM. Univariate or multivariate logistic regression analysis was performed to assess the association of albumin, GNRI, and CONUT with the diagnosis of sarcopenia. The optimal cut-off values were determined by the receiver operating characteristic (ROC) curve to diagnose sarcopenia.

Results: In 479 individuals with T2DM, the median age was 71 years [IQR 62, 77], including 264 (55.1%) men. The median duration of diabetes was 17 [11, 23] years. The prevalence of sarcopenia was 41 (8.6%) in all, 21/264 (8.0%) in men, and 20/215 (9.3%) in women. AUCs were ordered from largest to smallest as follows: GNRI > albumin > CONUT. The cut-off values of GNRI were associated with a diagnosis of sarcopenia in multiple logistic regression analysis (odds ratio 9.91, 95% confidential interval 5.72–17.2), $P < 0.001$. The superiority of GNRI as compared to albumin and CONUT for detecting sarcopenia was also observed in the subclasses of men, women, body mass index (BMI) < 22, and BMI ≥ 22.

Conclusions: Results showed that GNRI shows a superior diagnostic power in the diagnosis of sarcopenia. Additionally, its optimal cut-off points were useful overall or in the subclasses. Future large and prospective studies will be required to confirm the utility of the GNRI cut-off for undernutrition individuals at risk for sarcopenia.

KEYWORDS

aging, nutritional assessment, sarcopenia, type 2 diabetes, undernutrition

1. Introduction

Sarcopenia is a progressive and generalized skeletal muscle disorder involving the accelerated loss of muscle mass and function (1–3). Type 2 diabetes mellitus (T2DM) is associated with an increased risk of sarcopenia (4–7), which can increase adverse outcomes, including functional decline, frailty, falls, and mortality (8, 9). Factors associated with sarcopenia in diabetes are age, HbA1c levels, visceral obesity, diabetic nephropathy, duration of diabetes, and chronic inflammation (5–7).

Malnutrition/undernutrition can be defined as "a state resulting from lack of intake or uptake of nutrition that leads to altered body composition (decreased fat-free mass) and body cell mass leading to diminished physical and mental function and impaired clinical outcome from disease" (10). In older adults with diabetes, irregular and unpredictable meal consumption can be linked to undernutrition (11). Also, therapeutic diets or the use of anti-diabetic agents may inadvertently lead to decreased food intake and contribute to unintentional weight loss and undernutrition (11). Malnutrition/undernutrition can increase the risk of sarcopenia in older adults with diabetes (12–14). Undernutrition is a nutritional disorder, whereas sarcopenia and frailty are nutrition-related conditions with complex and multiple pathogenic backgrounds (10). Therefore, undernutrition should be diagnosed, and optimal nutritional intervention combined with an exercise program needs to be considered to prevent sarcopenia (11–14). However, there are few reports evaluating the relationship between undernutrition and the risk of sarcopenia in individuals with diabetes (12–14). If we could predict sarcopenia by screening undernutrition, we can manage such individuals through an optimal nutritional and exercise program.

The Geriatric Nutritional Risk Index (GNRI) is a nutritional screening index which had been proposed to assess the nutrition-related risk originally for hospitalized elderly by Bouillanne et al. (15). The GNRI is a simple and objective index, allowing clinicians to assess patients readily based on height, weight and serum albumin level. GNRI is currently known as a prognostic predictor for patients with chronic diseases such as cardiovascular disease (16), chronic kidney diseases (17) or cancer (18). The Controlling Nutritional Status (CONUT) score, which is calculated based on the serum albumin level, total peripheral lymphocyte count and total cholesterol level, was developed as a screening tool for early detection of poor nutritional status (19). The GNRI and CONUT are often used in clinical practice because they are simpler than other nutritional indicators such as SGA (Subjective Global Assessment), MNA(Mini Nutritional Assessment), MUST (Malnutrition Universal Screening Tool), and NRS2002 (Nutritional Risk Screening) which require an interview of an expert (physicians, nurses, and/or dieticians) (20). Although there are previous reports between GNRI and sarcopenia in T2DM (13, 21), the clinical utility has not been clarified.

Therefore, we investigated whether undernutritional status as assessed by GNRI (15, 21, 22) and CONUT (19) are associated with the diagnosis of sarcopenia and its components. We also evaluated how the cut-off values of these screening tools could detect sarcopenia in Japanese individuals with T2DM.

2. Methods

2.1. Study design and subjects

This is a cross-sectional study in the part of the Fukushima Diabetes, Endocrinology, and Metabolism cohort (Fukushima DEM cohort). The DEM cohort recruited people with diabetes mellitus or high risk at diabetes who had visited the Department of Diabetes, Endocrinology, and Metabolism, Fukushima Medical University Hospital. The study protocol was approved by the Fukushima Medical University Ethics Committee (Number 29118). This study was conducted according to the Ethical Guidelines for Medical and Health Research Involving Human Subjects enacted by MHLW of Japan (https://www.mhlw.go.jp/file/06-Seisakujouhou-10600000Daijinkanboukouseikagakuka/0000069410.pdf and http://www.mhlw.go.jp/file/06-Seisakujouhou-10600000-Daijinkanboukouseikagakuka/0000080278.pdf) in line with the principles of the Declaration of Helsinki. The inclusion criteria of the current study were people with T2DM in the DEM cohort who had been recruited between January 2018 and December 2019. Among 795 patients who gave written informed consent, 240 patients who were either non-diabetic, had type 1 diabetes mellitus, or had secondary diabetes mellitus were excluded from the study (Supplementary Figure 1). After excluding missing data, 479 patients (265 men and 215 women) were included for a full analysis set. Various patient parameters, such as age, sex, history of diabetes, family and social history, medical checkup history, complications, medications, laboratory data, and all dates, were obtained from their paper and/or electrical medical records.

2.2. Data collection

Patients visited the hospital at 1–3 month intervals and continued receiving standardized treatment by endocrinologists/diabetologists. Trained staff measured the height, body weight, blood pressure, and waist circumference of participants. Questionnaires were provided to record the data on smoking (current or former smoker or not), drinking (former or every day, sometimes, rarely, or never), regular exercise (exercise to sweat lightly for over 30 min on each occasion, two times weekly), antihypertensive drug use, anti-hyperglycemic drug use, and lipid-lowering drug use. A participant was diagnosed with diabetes mellitus when the fasting plasma glucose level is $\geq$126 mg/dL, the HbA1c level is $\geq$6.5% (48 mmol/mol), or if the participant regularly uses anti-hyperglycemic drugs. A participant was diagnosed with hypertension if the systolic blood pressure was $\geq$140 mmHg, if the diastolic blood pressure was $\geq$90 mmHg, or if she/he regularly used antihypertensive drugs. A participant was diagnosed with dyslipidemia if the high-density lipoprotein (HDL) cholesterol level is <40 mg/dL (1.0 mmol/L), the low-density lipoprotein (LDL) cholesterol level is $\geq$140 mg/dL (3.6 mmol/L), the triglyceride level is $\geq$150 mg/dL (1.7 mmol/L), or if they regularly used lipid-lowering drugs. We calculated the estimated glomerular filtration rate (eGFR) using the Japanese formula (eGFR, mL/min/1.73 m^2) = 194 × serum creatinine level (mg/dL) $^{-1.094}$ × age (years) $^{-0.287}$ (23).

Routine anthropometry and skeletal muscle mass, handgrip strength, walking speed, and body composition of the participants were assessed by trained staff, as previously reported (24). The

waist circumference was measured at the level of the umbilicus (cm) in the standing position. Handgrip strength (kg) was measured using an isokinetic dynamometer (Smedley hand dynamometer) on both hands, and the values of the non-dominant arm were used. The fat and muscle composition in the whole body, trunk, arms, and legs were assessed using a body composition analyzer (InBody 770, InBody Japan Inc.) based on the segmental multifrequency bioelectrical impedance analysis (25, 26). The time required for walking 10 m was measured as described previously with slight modifications (27, 28). Fasting blood samples were collected after overnight fasting for ≥10 h and were assayed within 1 h using automatic clinical chemical analyzers. We excluded participants whose fasting blood samples could not be obtained. Nutritional intake indices were calculated using food frequency questionnaires as previously reported (24).

2.3. Nutritional assessment tool

2.3.1. GNRI

The GNRI was calculated using the formula: GNRI = [14.89 × serum albumin level (g/dL)] + {41.7 × [current body weight (kg)/ideal body weight (kg)]} (15). In this study, the ideal body weight was determined from the participant's height and a BMI of 22 kg/m^2. Following previous studies (22), the participants were separated into two groups by a cut-off of GNRI 98 which is a commonly used diagnostic level for undernutrition (GNRI < 98 or GNRI ≥ 98). Based on the calculation of a GNRI cut-off point for detecting sarcopenia, we also subdivided the participants into GNRI < 105 or GNRI ≥ 105 groups.

2.3.2. CONUT

According to Ignacio de Ulíbarri J et al. (19), the CONUT score was obtained based on serum albumin concentration, cholesterol level, and lymphocyte count (Supplementary Table 1).

2.4. Assessment of sarcopenia

The definition and diagnosis of sarcopenia were based on the Asian Working Group for Sarcopenia (AWGS): 2019 Consensus Update on Sarcopenia Diagnosis and Treatment (2). In brief, "low muscle power" was defined as handgrip strength < 28 kg for men and <18 kg for women; the criteria for "low physical performance" was walking speed < 1.0 m/s as evaluated by the time required for walking 10 m; and "low appendicular skeletal muscle mass (ASM)" was defined as a skeletal mass index (SMI) < 7.0 kg/m^2 in men and <5.7 kg/m^2 in women. Sarcopenia is defined by low ASM and low muscle power or low physical performance.

3. Statistical analyses

Continuous and parametric values were expressed as mean ± standard deviation, and non-parametric values were expressed as median (first quartile–third quartile). Kolmogorov-Smirnov test was performed for normality. Group differences were analyzed by using two-tailed unpaired Student's *t*-test, one-way ANOVA or the Kruskal–Wallis test. Categorical values were expressed as percentages, and group differences were analyzed using the $\chi 2$ test.

We assessed the diagnostic value of serum albumin, BMI, GNRI, and CONUT by constructing the receiver operating characteristic (ROC) curve to distinguish between sarcopenia and non-sarcopenia in all participants, in men and women, in participants with BMI < 22 and BMI ≥ 22 and in diabetes duration < 5 and ≥ 5 years. The relevant area under the curve (AUC) was computed and compared as proposed by DeLong et al. (29). The optimal cut-off values were determined according to Youden's index, with the corresponding sensitivity, specificity, and accuracy at the cut-off value calculated and compared using the McNemar χ^2 test.

Univariate or multivariate logistic regression analysis was performed to assess the association of GNRI with sarcopenia and its components indicated by the odds ratio (OR) with 95% confidential intervals (CI) in the unadjusted or adjusted models, respectively. The selection of covariates in the multivariate analysis was based on the items strongly associated with sarcopenia in previous studies (1–7). Model 1 was adjusted for age (year) and sex; model 2 was further adjusted for model 2 plus diabetes duration (year), HbA1c (%), and eGFR (ml/min/1.73 m^2). Variables considered to have clinical implications were treated as potential variables to be controlled in model 3. BMI is a strong predictor of sarcopenia (1–7). Therefore, there was a risk of multicollinearity if BMI was included in the GNRI, which used current/ideal weight or current BMI/ BMI22 in the formula. When BMI and GNRI were simultaneously included in the multivariate logistic regression analysis to estimate sarcopenia, the VIF of BMI was 6.89 and the VIF of GNRI was 7.36, indicating a potential multicolineality with a VIF ≥ 5 (Supplementary Table 2). We therefore deleted BMI in the multivariate model using GNRI.

Statistical analyses were conducted using SPSS version 25 (SPSS, Inc., Chicago, Illinois, USA), EZR, or R (version 4.0.3). Values of $P < 0.05$ were considered statistically significant.

4. Results

4.1. General characteristics

4.1.1. Men vs. women

The demographic and clinical characteristics of 479 types 2 diabetes (264 men and 215 women) are shown in Table 1. The median age was 71 years [62, 77], and 55.1% of the patients were men. The median duration of diabetes is 17 [11, 23] years. The prevalence of sarcopenia was 41/479 (8.6%) in all, 21/264 (8.0%) in men, and 20/215 (9.3%) in women. Men were older and had a longer duration of diabetes. Moreover, men had lower BMI and systolic blood pressure. In nutritional indices, men had a slightly lower GNRI but showed comparable values to women in the other undernutrition indices, such as GNRI < 98, GNRI < 105, CONUT, and CONUT ≥ 2. In the indices for sarcopenia, men had higher values in SMI, handgrip strength, and walking speed and showed lower frequencies of low handgrip strength and low walking speed. However, men showed comparable frequencies in low SMI and sarcopenia. Regarding comorbidities, the prevalence of coronary heart disease was higher in men, but hypertension, dyslipidemia, and stroke were comparable. The ratio of regular waking, smoking, and drinking was higher in men.

TABLE 1 General characteristics of men and women with type 2 diabetes mellitus.

Parameters	All $n = 479$	Men $n = 264$	Women $n = 215$	*P*
Age, years	71 [62, 77]	72 [64, 77]	69 [60, 76]	0.038
Men, *n* (%)	264 (55.1)	–	–	
Duration of diabetes, years	17 [11, 23]	18 [11, 24]	15 [9, 21]	0.008
Anthropometry				
Body weight, kg	65.1 [56.3, 77.9]	67.2 [59.1, 79.4]	61.2 [51.0, 74.5]	<0.001
BMI, kg/m^2	25.2 [22.2, 29.3]	24.6 [22.0, 28.1]	26.4 [22.8, 31.0]	0.002
Systolic blood pressure, mmHg	132 ± 18	131 ± 17	134 ± 18	0.028
Diastolic blood pressure, mmHg	73 ± 12	74 ± 12	73 ± 11	0.153
Nutritional indices				
GNRI, points	111 [105, 119]	110 [105, 117]	112 [106, 120]	0.009
Range	85–167	85–167	93–166	
GNRI < 98, *n* (%)	43 (9.0)	26 (9.8)	17 (7.9)	0.460
GNRI < 105, *n* (%)	111 (23.2)	65 (24.6)	46 (21.4)	0.405
CONUT, points	1.0 [0.0, 2.0]	1.0 [0.0, 2.0]	1.0 [0.0, 2.0]	0.124
Range	0–6	0–6	0–5	
CONUT ≥ 2, *n* (%)	210 (60.9)	105 (57.4)	105 (64.8)	0.158
Indices for sarcopenia				
Skeletal muscle index, kg/m^2	7.1 [6.3, 7.9]	7.5 [7.0, 8.3]	6.4 [5.8, 7.1]	<0.001
Handgrip strength, kg	30 [22.5, 39.0]	38 [31.5, 43.0]	23 [18.5, 27.0]	<0.001
Walking speed, m/s	1.54 [1.33, 1.82]	1.67 [1.43, 1.82]	1.54 [1.25, 1.67]	<0.001
Low SMI, *n* (%)	100 (20.9)	60 (22.7)	40 (18.6)	0.270
Low handgrip strength, *n* (%)	85 (17.7)	34 (12.9)	51 (23.7)	0.002
Low walking speed, *n* (%)	22 (4.6)	7 (2.7)	15 (7.0)	0.024
Sarcopenia, *n* (%)	41 (8.6)	21 (8.0)	20 (9.3)	0.600
Comorbidities				
Retinopathy, *n* (%)	130 (27.1)	77 (29.2)	53 (24.7)	0.269
eGFR < 60 ml/min/1.73 m^2, *n* (%)	212 (44.3)	117 (44.3)	95 (44.2)	0.977
Hypertension, *n* (%)	403 (84.2)	223 (84.5)	180 (83.7)	0.823
Dyslipidemia, *n* (%)	409 (85.4)	218 (82.6)	191 (88.8)	0.054
Coronary heart disease, *n* (%)	74 (15.4)	56 (21.2)	18 (8.4)	<0.001
Stroke, *n* (%)	40 (8.4)	26 (9.8)	14 (6.5)	0.189
Life habits				
Regular walking, *n* (%)	118 (24.8)	76 (29.0)	42 (19.6)	0.018
Current or ex-smoking, *n* (%)	254 (53.0)	202 (76.5)	52 (24.2)	<0.001
Current or ex-drinking, *n* (%)	143 (29.9)	163 (61.7)	41 (19.1)	<0.001
Blood measurements				
Albumin, g/dL	4.2 [4.0, 4.4]	4.3 [4.1, 4.5]	4.2 [4.0, 4.4]	0.083
AST, U/L	21 [17, 28]	22 [17, 29]	20 [17, 26]	0.014
ALT, U/L	19 [14, 29]	21 [14, 32]	17 [13, 26]	0.002
Fasting plasma glucose, mg/dL	131 [118, 154]	138 [122, 159]	127 [113, 145]	<0.001
Glycated hemoglobin, %	6.9 [6.4, 7.4]	7.0 [6.5, 7.6]	6.8 [6.4, 7.3]	<0.001

(Continued)

TABLE 1 (Continued)

Parameters	All $n = 479$	Men $n = 264$	Women $n = 215$	P
LDL-cholesterol, mg/dL	100 [82, 118]	97 [80, 115]	104 [85, 124]	<0.001
HDL cholesterol, mg/dL	54 [46, 63]	51 [43, 60]	58 [49, 67]	<0.001
Triglycerides, mg/dL	105 [73, 153]	108 [73, 162]	102 [72, 141]	0.247
Creatinine, mg/dl	0.83 [0.69, 1.00]	0.92 [0.79, 1.10]	0.70 [0.60, 0.84]	<0.001
eGFR, ml/min/1.73 m^2	63.2 [51.0, 76.0]	62.8 [51.0, 75.6]	64.1 [51.0, 76.2]	0.828
Glucose-lowering drugs				
Insulin, *n* (%)	132 (27.6)	76 (28.8)	56 (26.0)	0.504
GLP-1 receptor agonist, *n* (%)	34 (7.1)	26 (9.8)	8 (3.7)	<0.001
Sulfonylurea, *n* (%)	49 (10.2)	34 (12.9)	15 (7.0)	0.034
Glinide, *n* (%)	118 (24.6)	76 (28.8)	42 (19.5)	0.019
Biguanide, *n* (%)	239 (49.9)	127 (48.8)	112 (52.1)	0.385
DPP4 inhibitor, *n* (%)	294 (61.4)	165 (62.5)	129 (60.0)	0.576
Pioglitazone, *n* (%)	147 (30.7)	82 (31.1)	65 (30.2)	0.845
α-glucosidase inhibitor, *n* (%)	90 (18.8)	63 (23.9)	27 (12.6)	0.002
SGLT2 inhibitor, *n* (%)	100 (20.9)	62 (23.5)	38 (17.7)	0.120
Nutritional intake				
Total energy intake (kcal/day)	2,010 [1,799–2,239]	2,212 [2,085–2,349]	1,795 [1,771–1825]	<0.001
Total protein intake (g/day)	78.0 [70.3–85.2]	84.3 [80.4–88.6]	69.7 [67.7–72.6]	<0.001
Total fat intake (g/day)	58.1 [54.7–63.6]	62.8 [59.8–66.5]	54.6 [53.8–55.8]	<0.001
Total carbohydrate intake (g/day)	259 [244–295]	245 [238–249]	293 [269–312]	<0.001
Protein energy (%)	15.4 [15.1–15.8]	15.3 [14.7–15.7]	15.6 [15.3–15.9]	<0.001
Fat energy (%)	27.1 [25.6–27.5]	25.7 [24.7–26.8]	27.4 [27.2–27.7]	<0.001

Data are expressed as median [25%, 75%], Mean ± SD, or number (%). GNRI, geriatric nutritional risk index; CONUT, controlling nutritional status; P, provability; BMI, body mass index; SMI, skeletal mass index; AST, aspartate aminotransferase; ALT, alanine aminotransferase; LDL, low-density lipoprotein; HDL, high-density lipoprotein; eGFR: estimated glomerular filtration rate; GLP-1, glucagon-like peptide-1; SGLT2, sodium-glucose cotransporter 2.

In blood measurements, albumin and eGFR were comparable between men and women. On the other hand, AST, ALT, glucose, HbA1c, and triglycerides were higher in men, and LDL- and HDL cholesterol were lower in men. Regarding anti-diabetic medication, the use of GLP-1 receptor agonist, sulfonylurea, glinide, and α-glucosidase inhibitor was higher in men, but the use of the other anti-diabetic medications was comparable.

4.1.2. Sarcopenia− vs. sarcopenia+

The general characteristics of participants in the subgroups according to sarcopenia are shown in Table 2, left panel. The sarcopenia+ groups were older and had a longer duration of diabetes, lower BMI, and lower diastolic blood pressure. There was no difference in the ratios of men. As shown in Table 2 and Figure 1 (upper panel), the sarcopenia+ group showed a lower GNRI and higher frequencies in GNRI < 98 and GNRI < 105 while showing no difference in CONUT indices. The frequencies of comorbidities, except stroke, were comparable. Blood measurements and the use of glucose-lowering drugs were comparable, except that albumin and LDL-cholesterol were lower in the sarcopenia+ group. As shown in Figure 1 (middle panel), the sarcopenia+ groups in men and women showed lower values in BMI, albumin, and GNRI. Nutritional intake including total energy intake, total protein intake, total fat intake, total carbohydrate intake, protein energy, fat energy, and carbohydrate energy were not different between sarcopenia− vs. sarcopenia+.

4.1.3. GNRI < 98 vs. GNRI ≥ 98

The group with GNRI < 98 was older and had a longer duration of diabetes, lower BMI, and lower systolic and diastolic blood pressure (Supplementary Table 3). There was no difference in terms of sex. They also showed a lower GNRI and a higher CONUT. Regarding sarcopenia, the group with GNRI < 98 had lower SMI, handgrip strength, higher frequencies of low SMI and low handgrip strength, and higher sarcopenia (30.2 vs. 6.4%, $P < 0.001$). They also showed lower values in albumin, ALT, LDL-cholesterol, and triglycerides. The use of α-glucosidase inhibitor was higher, and that of SGLT2 inhibitor was lower in this group.

4.1.4. GNRI < 105 vs. GNRI ≥ 105

As described below, we found that GNRI < 105 was the cut-off for detecting sarcopenia in our participants. Therefore, we compared two subgroups accordingly (Table 2). The GNRI < 105 participants were

TABLE 2 General characteristics of participants with type 2 diabetes mellitus in the subgroups accordingly to sarcopenia, GNRI, and CONUT.

Parameters	Sarcopenia − $n = 438$	Sarcopenia + $n = 41$	*P*	GNRI < 105 $n = 111$	GNRI ≥ 105 $n = 368$	*P*	CONUT < 2 $n = 210$	CONUT ≥ 2 $n = 135$	*P*
Age, years	70 [60, 75]	80 [74, 86]	<0.001	73 [70, 79]	69 [59, 75]	<0.001	68 [57, 74]	72 [63, 79]	0.003
Men, *n* (%)	243 (55.5)	21 (51.2)	0.600	65 (58.6)	199 (54.1)	0.405	105 (50.0)	78 (57.8)	0.158
Duration of diabetes, years	16 [11, 22]	21 [14, 28]	0.013	18 [11, 26]	16 [11, 22]	0.041	15 [9, 21]	18 [12, 24]	0.006
Anthropometry									
Body weight, kg	66.6 [58.1, 79.5]	51.8 [46.7, 56.7]	<0.001	53.3 [47.7, 58.9]	69.4 [60.9, 81.9]	<0.001	67.4 [57.6, 82.9]	63.6 [54.5, 77.1]	0.016
BMI, kg/m^2	25.7 [22.8, 29.6]	22.0 [20.5, 23.9]	<0.001	20.9 [19.8, 21.9]	27.1 [24.3, 30.8]	<0.001	26.5 [23.3, 31.0]	25.3 [21.9, 28.3]	0.011
Systolic blood pressure, mmHg	132 ± 17	130 ± 19	0.550	131 [118, 142]	132 [121, 143]	0.133	133 ± 18	133 ± 16	0.764
Diastolic blood pressure, mmHg	74 ± 12	67 ± 10	<0.001	71 [64, 79]	74 [67, 82]	0.005	74 ± 12	74 ± 11	0.673
Nutritional indices									
GNRI	112 [106, 120]	101 [96, 106]	<0.001	100 [96, 102]	115 [110, 122]	<0.001	114 [106, 122]	109 [102, 115]	<0.001
Range	85–167	87–118		85–104	105–167		93–166	85–167	
GNRI < 98, *n* (%)	30 (6.8)	13 (31.7)	<0.001	43 (38.7)	0 (0)	<0.001	15 (7.1)	20 (14.8)	0.021
GNRI < 105, *n* (%)	85 (19.4)	26 (63.4)	<0.001				43 (20.5)	38 (28.1)	0.101
CONUT, points	1.0 [0.0, 2.0]	1.0 [1.0, 3.0]	0.281	1.0 [0.5, 2.5]	1.0 [1.0, 2.0]	0.107	1.0 [0.0, 1.0]	2.0 [2.0, 3.0]	<0.001
Range	0–6	0–4		0–6	0–5		0–1	2–6	
CONUT ≥ 2, *n* (%)	122 (38.7)	13 (43.3)	0.622	38 (46.9)	97 (36.7)	0.101			
Indices for sarcopenia									
Skeletal muscle index, kg/m^2	7.2 [6.4, 8.0]	5.6 [5.3, 6.4]	<0.001	6.3 [5.6, 6.9]	7.4 [6.5, 8.1]	<0.001	7.2 [6.3, 8.1]	7.0 [6.2, 7.9]	0.277
Handgrip strength, kg	31.5 [24.0, 39.5]	17.5 [14.5, 22.3]	<0.001	26.0 [19.0, 33.0]	31.5 [23.5, 40.4]	<0.001	29.3 [22.0, 40.0]	29.0 [22.5, 37.5]	0.679
Walking speed, m/s	1.54 [1.43, 1.82]	1.25 [1.11, 1.43]	<0.001	1.54 [1.33, 1.67]	1.54 [1.33, 1.82]	0.286	1.54 [1.33, 1.67]	1.54 [1.33, 1.67]	0.747
Low SMI, *n* (%)	59 (13.5)	41 (100)	<0.001	24 (55.8)	76 (17.4)	<0.001	36 (17.1)	33 (24.4)	0.098
Low handgrip, *n* (%)	44 (10.0)	41 (100)	<0.001	33 (29.7)	52 (14.1)	<0.001	35 (16.7)	31 (23.0)	0.147
Low walking speed, *n* (%)	16 (3.7)	6 (14.6)	0.001	5 (4.5)	17 (4.6)	0.960	10 (4.8)	7 (5.2)	0.859
Sarcopenia, *n* (%)	0 (0)	41 (100)		26 (23.4)	15 (4.1)	<0.001	17 (8.1)	13 (9.6)	0.622
Comorbidities									
Retinopathy, *n* (%)	119 (27.2)	11 (26.8)	0.120	35 (31.5)	95 (25.9)	0.241	55 (26.2)	37 (27.4)	0.803
eGFR< 60 ml/min/1.73m^2, *n* (%)	191 (43.6)	21 (51.2)	0.348	49 (44.1)	163 (44.4)	0.960	84 (40.0)	70 (51.9)	0.031

(Continued)

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TABLE 2 (Continued)

Parameters	Sarcopenia − $n = 438$	Sarcopenia + $n = 41$	P	GNRI < 105 $n = 111$	GNRI ≥ 105 $n = 368$	P	CONUT < 2 $n = 210$	CONUT ≥ 2 $n = 135$	P
Hypertension, *n* (%)	370 (84.5)	33 (80.5)	0.504	80 (72.1)	323 (87.7)	<0.001	177 (84.3)	117 (86.7)	0.543
Dyslipidemia, *n* (%)	374 (85.4)	35 (85.4)	0.997	78 (70.3)	331 (89.9)	<0.001	182 (86.7)	113 (83.7)	0.445
Coronary heart disease, *n* (%)	68 (15.5)	6 (14.6)	0.880	11 (9.9)	63 (17.1)	0.065	25 (11.9)	23 (17.0)	0.179
Stroke, *n* (%)	33 (7.5)	7 (17.1)	0.035	8 (7.2)	32 (8.7)	0.619	17 (8.1)	11 (8.1)	0.986
Life habits									
Regular walking, *n* (%)	111 (25.5)	7 (17.5)	0.265	27 (24.8)	91 (24.8)	0.996	46 (22.1)	25 (18.7)	0.441
Current or ex-smoking, *n* (%)	235 (53.7)	19 (46.3)	0.370	19 (46.3)	235 (53.7)	0.370	114 (54.3)	67 (49.6)	0.398
Current or ex-drinking, *n* (%)	190 (43.4)	14 (34.1)	0.253	14 (34.1)	190 (43.4)	0.253	82 (39.0)	60 (44.4)	0.320
Blood measurements									
Albumin, g/dL	4.3 [4.1, 4.5]	4.0 [3.8, 4.3]	<0.001	4.1 [3.8, 4.2]	4.3 [4.1, 4.5]	<0.001	4.3 [4.1, 4.5]	4.2 [3.9, 4.4]	0.004
AST, U/L	21 [17, 28]	21 [18, 26]	0.779	21 [17, 27]	21 [17, 28]	0.683	20 [17, 28]	22 [18, 29]	0.057
ALT, U/L	19 [14, 29]	16 [13, 23]	0.064	16 [11, 23]	20 [14, 31]	<0.001	19 [14, 30]	18 [12, 30]	0.172
Fasting plasma glucose, mg/dL	131 [118, 154]	130 [117, 150]	0.842	129 [114, 151]	132 [118, 154]	0.223	131 [117, 154]	132 [120, 155]	0.658
Glycated hemoglobin, %	6.9 [6.4, 7.5]	6.8 [6.3, 7.4]	0.429	6.8 [6.4, 7.4]	6.9 [6.4, 7.5]	0.747	6.9 [6.4, 7.6]	6.9 [6.4, 7.4]	0.172
LDL-cholesterol, mg/dL	102 [84, 119]	86 [78, 107]	0.024	97 [81, 114]	102 [83, 120]	0.105	110 [94, 129]	87 [75, 103]	<0.001
HDL cholesterol, mg/dL	54 [46, 62]	51 [43, 71]	0.940	55 [44, 70]	53 [46, 62]	0.097	56 [46, 64]	50 [44, 62]	0.011
Triglycerides, mg/dL	104 [73, 154]	105 [70, 147]	0.531	82 [60, 129]	109 [78, 160]	<0.001	117 [84, 179]	90 [66, 132]	<0.001
Creatinine, mg/dl	0.84 [0.69, 1.01]	0.80 [0.68, 0.97]	0.409	0.83 [0.68, 1.03]	0.83 [0.69, 0.99]	0.898	0.82 [0.67, 1.00]	0.84 [0.71, 1.06]	0.117
eGFR, ml/min/1.73 m^2	63.3 [51.1, 76.2]	59.9 [49.7, 73.6]	0.491	62.7 ± 19.7	63.3 ± 17.8	0.663	64.9 [50.9, 77.6]	59.5 [48.8, 74.9]	0.092
Glucose-lowering drugs									
Insulin, *n* (%)	121 (27.6)	11 (26.8)	0.913	43 (38.7)	89 (24.2)	0.003	53 (25.2)	45 (33.3)	0.104
GLP-1 receptor agonist, *n* (%)	34 (7.8)	0 (0)	0.064	4 (3.6)	30 (8.2)	0.102	15 (7.1)	9 (6.7)	0.865
Sulfonylurea, *n* (%)	43 (9.8)	6 (14.6)	0.330	12 (10.8)	37 (10.1)	0.818	25 (11.9)	11 (8.1)	0.265
Glinide, *n* (%)	106 (24.2)	12 (29.3)	0.471	33 (29.7)	85 (23.1)	0.155	46 (21.9)	31 (23.0)	0.818
Biguanide, *n* (%)	221 (50.5)	18 (43.9)	0.422	46 (41.4)	193 (52.4)	0.042	104 (49.5)	62 (45.9)	0.514
DPP4 inhibitor, *n* (%)	268 (61.2)	26 (63.4)	0.779	67 (60.4)	227 (61.7)	0.802	123 (58.6)	84 (62.2)	0.499
Pioglitazone, *n* (%)	137 (31.3)	10 (24.4)	0.360	17 (15.3)	130 (35.7)	<0.001	64 (30.5)	43 (31.9)	0.787
α-glucosidase inhibitor, *n* (%)	79 (18.0)	11 (26.8)	0.168	29 (26.1)	61 (16.6)	0.024	40 (19.0)	17 (12.6)	0.115

(Continued)

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TABLE 2 (Continued)

Parameters	Sarcopenia − $n = 438$	Sarcopenia + $n = 41$	P	GNRI < 105 $n = 111$	GNRI ≥ 105 $n = 368$	P	CONUT < 2 $n = 210$	CONUT ≥ 2 $n = 135$	P
SGLT2 inhibitor, *n* (%)	94 (21.5)	6 (14.6)	0.304	15 (13.5)	85 (23.1)	0.029	47 (22.4)	27 (20.0)	0.599
Nutritional intake									
Total energy intake (kcal/day)	2,012 [1,802–2,243]	1,926 [1,781–2,184]	0.157	2,039 [1,796–2,228]	1,997 [1,799–2,240]	0.941	1,951 [1,787–2,210]	2,031 [1,800–2,278]	0.187
Total protein intake (g/day)	78.2 [70.5–85.3]	76.9 [68.3–83.8]	0.323	78.7 [70.9–85.3]	77.7 [70.2–85.2]	0.549	77.0 [69.3–84.3]	79.0 [70.6–85.4]	0.133
Total fat intake (g/day)	58.2 [54.8–63.6]	57.3 [54.1–62.9]	0.404	59.3 [54.8–63.8]	58.0 [54.7–63.6]	0.639	57.3 [51.9–−54.8]	59.2 [55.3–63.7]	0.090
Total carbohydrate intake (g/day)	260 [245–296]	250 [241–292]	0.170	257 [243–297]	260 [245–295]	0.700	255 [242–293]	262 [246–302]	0.165
Protein energy (%)	15.4 [15.1–15.8]	15.5 [15.2–15.7]	0.756	15.5 [15.1–15.9]	15.4 [15.1–15.8]	0.220	15.4 [15.1–15.7]	15.3 [15.1–15.8]	0.843
Fat energy (%)	27.0 [25.6–27.5]	27.3 [25.9–27.6]	0.172	27.2 [25.7–27.6]	27.0 [25.6–27.5]	0.616	27.2 [25.6–27.5]	27.0 [25.5–27.5]	0.956
Carbohydrate energy (%)	53.7 [52.0–55.0]	53.5 [51.8–54.6]	0.359	53.6 [51.2–54.7]	53.7 [52.0–55.0]	0.234	53.8 [52.4–55.0]	53.7 [51.9–54.8]	0.492

Data are expressed as median [25%, 75%], Mean ± SD, or number (%). GNRI, geriatric nutritional risk index; CONUT, controlling nutritional status; P, provability; BMI, body mass index; SMI, skeletal mass index; AST, aspartate aminotransferase; ALT, alanine aminotransferase; LDL, low-density lipoprotein; HDL, high-density lipoprotein; eGFR: estimated glomerular filtration rate; GLP-1, glucagon-like peptide-1; SGLT2,sodium-glucose cotransporter 2.

older and had a longer duration of diabetes, lower BMI, and lower diastolic blood pressure but showed a comparable value in systolic blood pressure (Table 2). Regarding sarcopenia, the GNRI < 105 participants, as well as the GNRI < 98 participants, had lower values in SMI, handgrip strength, higher frequencies of low SMI and low handgrip strength, and higher sarcopenia. The use of insulin and α-glucosidase inhibitor was higher, and that of pioglitazone and SGLT2 inhibitor was lower in this group.

4.1.5. CONUT < 2 vs. CONUT ≥ 2

The CONUT ≥ 2 group, which was estimated to be in an undernutrition state, was older and had a longer duration of diabetes and lower BMI but showed a comparable value in systolic and diastolic blood pressure as compared to the CONUT < 2 group (Table 2). However, the indices for sarcopenia were all comparable between the CONUT < 2 vs. CONUT ≥ 2 groups. The CONUT ≥ 2 group showed lower values in albumin, LDL- and HDL cholesterol, and triglycerides. The use of glucose-lowering drugs was similar between the two subgroups.

4.2. Diagnostic assessment of the nutritional indices for diagnosis of sarcopenia

The AUCs and the optimal cut-off values of albumin, GNRI, and CONUT for detecting sarcopenia are shown in Figure 2 and Table 3. In all participants, the AUCs were ordered from largest to smallest as follows: GNRI > albumin > CONUT, showing that the diagnostic power of GNRI was superior to albumin. The AUC of GNRI was also statistically significant and was superior to albumin in all, men, women, BMI ≥ 22, and diabetes duration ≥ 5 years subgroups (Figure 2; Table 3).

4.3. Univariate and multivariate logistic regression analysis on the associations of nutritional indices with the diagnosis of sarcopenia and its components

Based on the cut-off values of the nutritional indices, we calculated the ORs for the diagnosis of sarcopenia and its components. In all participants (Table 4), univariate and multiple logistic regression analysis showed that albumin was associated with low handgrip strength and sarcopenia but not with low SMI and low walking speed. The cut-off values of GNRI of 98 and 105 were associated with the diagnosis of low SMI, low handgrip strength, and sarcopenia in univariate and multiple logistic regression analysis (models 1 and 2). The cut-off value of CONUT was not associated with the diagnosis of sarcopenia and its components.

Multiple logistic regression analysis (Model 2 in Table 4) on the associations of nutritional indices with the diagnosis of sarcopenia and its components in the subclasses of current participants is shown in Table 5 (ORs in unadjusted and Model 1 not shown). The cut-off values of albumin were associated with a diagnosis of sarcopenia only in women but not in the men, BMI < 22 and BMI ≥ 22 subclasses. The cut-off values of GNRI were associated with a diagnosis of sarcopenia in the men (105), women (105), BMI <22 (102), BMI

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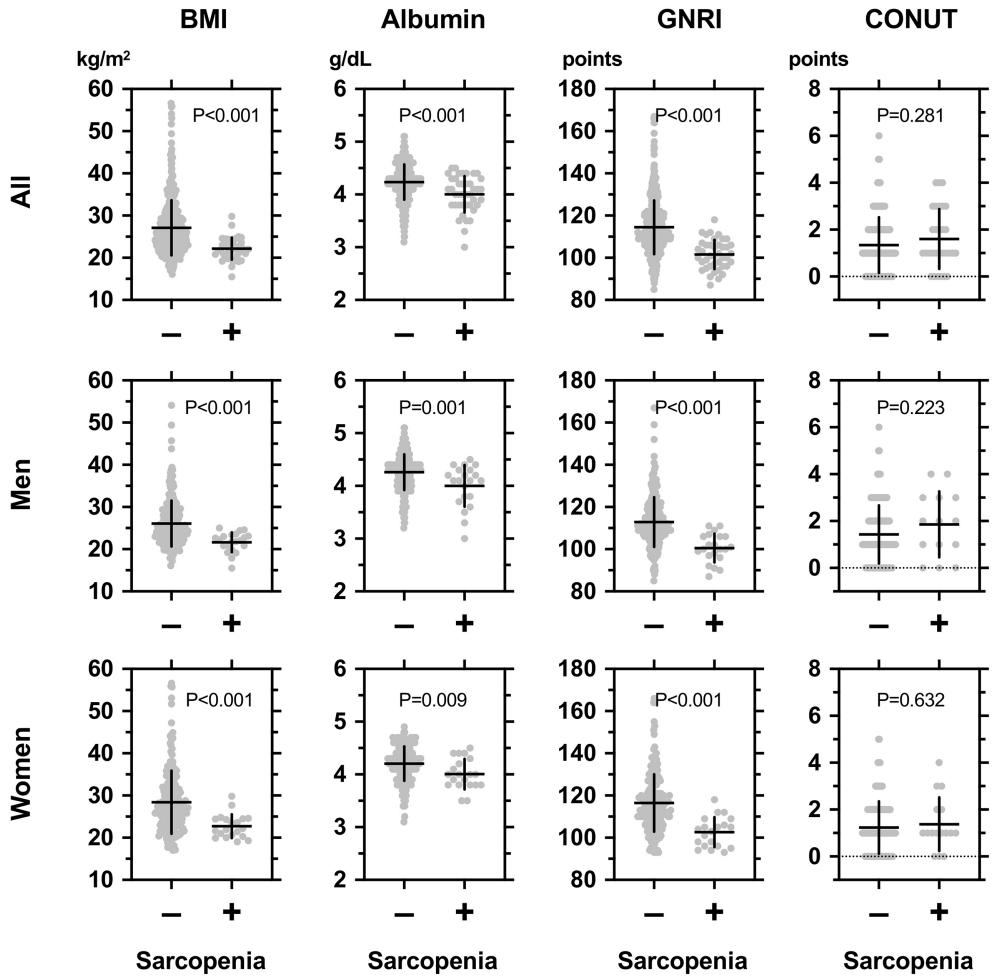

FIGURE 1
Comparisons among body mass index (BMI), serum albumin concentrations, geriatric nutritional risk index (GNRI), and controlling nutritional status (CONUT) between individuals with (+) or without (−) sarcopenia in all participants **(upper panel)**, men **(middle panel)**, and women **(lower panel)** with type 2 diabetes mellitus. The definition and diagnosis of sarcopenia were based on the Asian Working Group for Sarcopenia (AWGS): 2019 Consensus Update on Sarcopenia Diagnosis and Treatment (2). In brief, "low muscle power" was defined as handgrip strength < 28 kg for men and <18 kg for women; the criterion for "low physical performance" was walking speed < 1.0 m/s as evaluated by the time required for walking 10 m; and "low appendicular skeletal muscle mass (ASM)" was defined as a skeletal mass index (BMI) < 7.0 kg/m^2 in men and <5.7 kg/m^2 in women. Sarcopenia was defined by low skeletal mass index (SMI) and low muscle power or low physical performance.

≥ 22 (112), and diabetes duration ≥ 5 years (105) subgroups, but not in the diabetes duration < 5 years (Table 5). When using an originally reported low nutrition-related cut-off at 98 (15), the GNRI was associated with sarcopenia in the men, women, and diabetes duration ≥ 5 years subgroups but not in the BMI < 22, BMI ≥ 22, and diabetes duration < 5 years subgroups. The cut-off values of CONUT were not associated with sarcopenia and its components in these subclasses, except in patients with BMI < 22.

5. Discussion

The current study investigated whether undernutrition status, as assessed by GNRI, CONUT, and albumin, is associated with the diagnosis of sarcopenia and its components. We also determined the diagnostic power of the cut-off values for detecting sarcopenia in Japanese individuals with T2DM. We obtained two major findings. First, the cut-off values of albumin and GNRI 98 and 105, but not that of CONUT, were associated with a diagnosis of sarcopenia in the overall, men and women groups (Tables 4, 5). The AUC of GNRI was significantly larger than those of albumin and CONUT, indicating that the diagnostic power of GNRI was superior to both (Table 3). Second, the superiority of GNRI as compared to albumin and CONUT for sarcopenia was also observed in the subclasses. The AUCs of GNRI was significantly larger than that of albumin and CONUT in these subclasses (Table 3). The cut-off values of GNRI were associated with a diagnosis of sarcopenia in the BMI < 22 (102), BMI ≥ 22 (112), and diabetes duration ≥ 5 years (105) subgroups (Table 5). The GNRI cut-off of 98, which was commonly used as the diagnostic level for undernutrition (29), was not associated with sarcopenia in the BMI < 22 and BMI ≥ 22 subgroups. The cut-off values of albumin and CONUT were not associated with a diagnosis of sarcopenia.

To our knowledge, this study first provides us with a comparison of the diagnostic utility of the indexes commonly

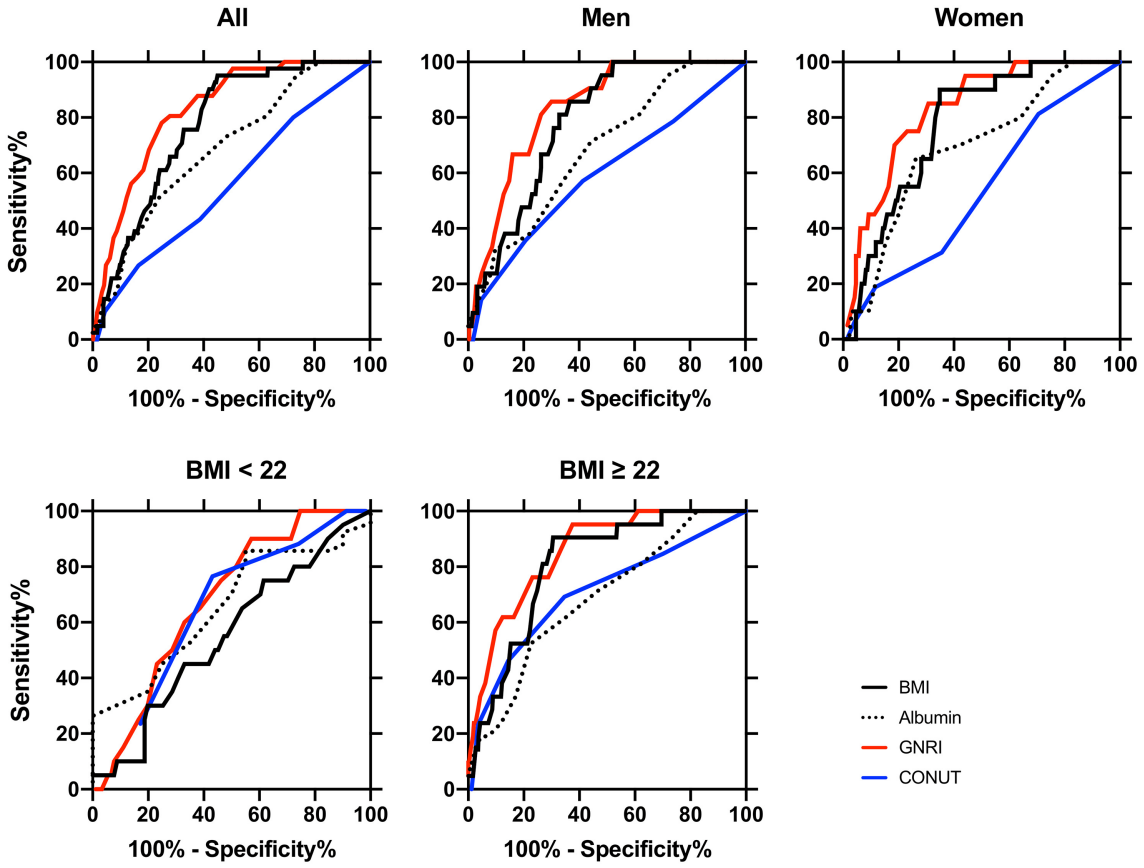

FIGURE 2
Receiver-operating characteristic (ROC) curves of the nutritional indexes for distinguishing between sarcopenia ($n = 41$) and non-sarcopenia ($n = 438$) patients in all participants ($n = 479$), in men ($n = 264$), in women ($n = 215$), BMI < 22 ($n = 111$), and BMI ≥ 22 ($n = 368$) subclasses. The area under the ROC curve (AUC) of body mass index (BMI, black lines), serum albumin (dotted lines), geriatric nutritional risk index (GNRI, red lines), and controlling nutritional status (CONUT, blue lines) were calculated for detecting diagnosis of sarcopenia, and statistical significance between AUC is shown in Table 3. The cut-off values, sensitivity, and specificity of these indices are also shown in Table 3. The definition and diagnosis of sarcopenia were based on the Asian Working Group for Sarcopenia (AWGS): 2019 Consensus Update on Sarcopenia Diagnosis and Treatment (2).

used in the nutritional assessment of people with T2DM. This study also determined the cut-off values of the nutritional indexes in sarcopenia and made a comparison to show their superiority or inferiority. We found that GNRI, a simple screening formula for undernutrition, shows a superior diagnostic power. Future large and prospective studies will be required to confirm the utility of the GNRI cut-off for undernutrition individuals at risk for sarcopenia.

5.1. GNRI and diagnosis of sarcopenia and its components

There are reports on the associations between the nutritional indicators such as SGA, MNA, MUST, and NRS2002 and diagnosis of sarcopenia (20, 30, 31). These reports repeatedly indicated that malnutrition determined by these indices and diagnosis of sarcopenia are closely linked (20, 30, 31). However, these indicators require interviews for history of body weight and dietary assessment, limiting clinical application. There are more simpler indices such as albumin and prealbumin. Xiu et al. reported that low prealbumin levels were associated with an increased risk for sarcopenia in older men with T2DM (32). However, prediction of undernutrition using these simple indices may be limited to some extent by potential confounding factors such as other clinical conditions (20, 30, 31). In our study, we adopted GNRI (15, 21, 22), CONUT (19), and albumin which are easily available in daily clinical practice for the assessment of undernutrition.

The cut-off values of GNRI were associated with a diagnosis of sarcopenia in multiple logistic regression analysis after correcting for potential cofounders. There are previous reports on the association between GNRI with the diagnosis of sarcopenia and its components. In Korean patients on hemodialysis, a GNRI of 97–101 (OR 0.064, 95% CI 0.005–0.883, compared to GNRI ≤ 96, $p = 0.040$) was associated with a lower sarcopenia risk (33). Xiang et al. reported that the overall diagnostic performance was the best for mid-arm circumference, followed by GNRI, calf circumference, BMI, and the worst for triceps skinfold thickness and albumin in detecting sarcopenia in community-dwelling Chinese adults aged 50 or older (34). The following two reports agreed with our findings, showing the association between low GNRI and the diagnosis of sarcopenia (13, 21). Takahashi et al. reported that a GNRI < 98

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TABLE 3 Diagnostic value of the nutritional indexes for distinguishing between sarcopenia and non-sarcopenia.

	AUC	95% CI	Cut-off	*P*	P. vs. albumin	*P* vs. GNRI
All						
Albumin, g/dL	0.687	(0.607–0.766)	4.05	<0.001	–	–
GNRI, points	0.827	(0.773–0.881)	105.8	<0.001	<0.001	–
CONUT, points	0.557	(0.448–0.667)	3.00	0.299	0.108	<0.001
Men						
Albumin, g/dL	0.683	(0.573–0.792)	4.20	0.006	–	–
GNRI, points	0.833	(0.763–0.903)	106.9	<0.001	<0.001	–
CONUT, points	0.592	(0.425–0.725)	2.00	0.256	0.481	0.010
Women						
Albumin, g/dL	0.688	(0.574–0.803)	4.000	0.006	–	–
GNRI, points	0.827	(0.749–0.905)	105.8	<0.001	0.012	–
CONUT, points	0.534	(0.390–0.677)	1.00	0.660	0.084	<0.001
BMI < 22						
Albumin, g/dL	0.655	(0.535–0.776)	3.80	0.030	–	–
GNRI, points	0.679	(0.568–0.790)	102.0	0.013	0.641	–
CONUT, points	0.654	(0.515–0.793)	1.00	0.055	0.673	0.729
BMI ≥ 22						
Albumin, g/dL	0.684	(0.572–0.797)	4.00	0.005	–	–
GNRI, points	0.852	(0.783–0.922)	112.1	<0.001	<0.001	–
CONUT, points	0.706	(0.544–0.869)	2.00	0.012	0.721	0.035
Diabetes duration < 5 years						
Albumin, g/dL	0.321	(0.037–0.606)	4.40	0.316	–	–
GNRI, points	0.786	(0.593–0.979)	109.2	0.109	0.062	–
CONUT, points	0.435	(0.186–0.684)	1.00	0.828	0.102	0.015
Diabetes duration ≥ 5 years						
Albumin, g/dL	0.711	(0.632–0.789)	4.00	<0.001	–	–
GNRI, points	0.831	(0.775–0.887)	105.8	<0.001	<0.001	–
CONUT, points	0.446	(0.332–0.559)	3.00	0.333	0.066	<0.001

AUC, area under the curve; GNRI, geriatric nutritional risk index; CONUT, controlling nutritional status; CI, confidential intervals; P, provability.

was related to the prevalence of sarcopenia [adjusted odds ratio, 4.88 (95%CI: 1.88–12.7), $p = 0.001$] in Japanese patients with T2DM (13). Matsuura et al. reported that a higher GNRI was associated with a lower risk of sarcopenia in older men and women with diabetes [multivariate-adjusted OR, 0.892; 95% CI, 0.839–0.948 for male; adjusted OR, 0.928; 0.876–0.982 for female] (21). However, the GNRI threshold and its relevance in subclasses for sarcopenia were not considered in the two studies. We further assessed the diagnostic utility of the cut-off values of GNRI to detect sarcopenia. The GNRI cut-off values of 105 and 98 were associated with a diagnosis of sarcopenia similarly in all participants and in the men and women subclasses. However, a GNRI of 102 in patients with BMI < 22 and a GNRI of 112 in patients with BMI ≥ 22, but not that of 98, were associated with a diagnosis of sarcopenia. Collectively, it is suggested that the optimal cut-off values of GNRI depend on the clinical characteristics of the target population.

5.2. Potential mechanisms by which GNRI predicts sarcopenia

There were reports indicating the association between low GNRI and low muscle power, and low muscle mass (22, 35). In Chinese elderly people, a low GNRI was associated with a higher incidence of low muscle mass (34). In Italian institutionalized elderly, GNRI was correlated with arm muscle area, handgrip strength, and handgrip strength/arm muscle area (22). Compared to other indices such as the ESPEN, GLIM, or SGA criteria, GNRI appears simple but still considers the serum albumin level in addition to current and ideal body weight (15).

The mechanisms by which low GNRI correlates with sarcopenia may include the lack of supply of muscle building blocks due to undernutrition and the involvement of chronic inflammation of muscle due to undernutrition (14, 36, 37). In Germany's older patients, a higher risk GNRI was associated with increased

TABLE 4 Univariate and multivariate logistic regression analysis on associations of cut-off of nutritional indices with a diagnosis of sarcopenia in the overall participants.

Dependent variable	Unadjusted OR (95% CI)	*P*	Model 1	*P*	Model 2	*P*
Independent variable: Albumin cut-off 4.05						
Low SMI	1.43 (0.88–2.31)	0.147	1.06 (0.63–1.79)	0.829	1.06 (0.61–1.83)	0.832
Low handgrip strength	3.84 (2.35–6.26)	<0.001	3.37 (1.93–5.89)	<0.001	3.73 (2.08–6.67)	<0.001
Low walking speed	2.45 (1.03–5.82)	0.042	1.64 (0.65–4.13)	0.294	2.09 (0.78–5.59)	0.141
Sarcopenia	3.33 (1.74–6.38)	<0.001	2.39 (1.18–4.86)	0.016	2.65 (1.26–5.56)	0.010
Independent variable: GNRI cut-off 98						
Low SMI	5.99 (3.12–11.47)	<0.001	4.26 (2.13–8.49)	<0.001	4.09 (2.02–8.27)	<0.001
Low handgrip strength	3.54 (1.82–6.87)	<0.001	2.56 (1.22–5.37)	0.013	2.60 (1.22–5.53)	0.013
Low walking speed	1.65 (0.47–5.80)	0.438	1.08 (0.29–4.05)	0.911	1.35 (0.35–5.19)	0.664
Sarcopenia	6.31 (2.97–13.44)	<0.001	4.64 (2.01–10.69)	<0.001	4.67 (1.98–11.01)	<0.001
Independent variable: GNRI cut-off 105						
Low SMI	11.74 (7.08–19.48)	<0.001	5.77 (2.74–12.1)	<0.001	0.75 (0.30–1.86)	<0.001
Low handgrip strength	2.57 (1.56–4.25)	<0.001	1.84 (1.05–3.23)	0.033	1.84 (1.04–3.25)	0.035
Low walking speed	0.97 (0.35–2.70)	0.960	0.62 (0.21–1.82)	0.388	0.66 (0.22–1.99)	0.457
Sarcopenia	7.19 (3.65–14.18)	<0.001	5.77 (2.74–12.1)	<0.001	9.91 (5.72–17.2)	<0.001
Independent variable: CONUT cut-off 3.0						
Low SMI	1.60 (0.87–2.95)	0.131	1.50 (0.77–2.92)	0.240	1.59 (0.80–3.16)	0.190
Low handgrip strength	1.49 (0.78–2.86)	0.228	1.66 (0.79–3.50)	0.185	1.78 (0.83–3.79)	0.138
Low walking speed	1.59 (0.52–4.87)	0.416	1.78 (0.54–5.92)	0.348	1.70 (0.49–5.86)	0.400
Sarcopenia	1.80 (0.79–4.11)	0.163	1.76 (0.71–4.40)	0.226	1.81 (0.70–4.68)	0.222

Model 1: Age (year) and sex.
Model 1: Age (year) and sex Model 2: age (year), sex, diabetes duration (years), HbA1c (%), and eGFR (ml/min/1.73 m^2).
OR, odds ratio; SMI, skeletal mass index; GNRI, geriatric nutritional risk index; CONUT, controlling nutritional status; CI, confidential intervals; P, provability.

CRP levels ($p < 0.05$) and low lymphocyte counts ($p < 0.05$) after multivariable adjustment (36). Subclinical catabolic and inflammatory states, which are associated with chronic disease, led to increased production of catabolic cytokines, increased muscle catabolism, and decreased appetite with a negative effect on albumin levels (38–40). A reduction in serum albumin can therefore be a consequence of poor nutritional status or inflammation/disease (38–40).

Although low albumin has long been recognized as a crude indicator of undernutrition status (41), it is an unreliable indicator of nutritional status because it may be more related to inflammation or hydration status than to malnutrition (15, 42, 43). The GNRI was developed by Bouillanne et al. in 2005 to provide a prognostic nutritional index that enables quantitative determination of the risk of nutrition-related morbidity and mortality in elderly patients at admission into a geriatric hospital (15). They described that GNRI is not an index of malnutrition, but it is a "nutrition-related" risk index because GNRI scores are correlated to a severity score that considers nutritional status-related complications such as bedsores and infections (15). Importantly, GNRI is also based on measurements of weight loss, which are strong independent risk factors for comorbidities and mortality in older persons (44, 45). Applying the status of weight in the formula, GNRI can be a better predictor than serum albumin for low SMI and sarcopenia in the elderly with T2DM with a median age of 80 [IQR 74, 86]. The CONUT formula includes blood biomarkers such as serum albumin concentration, cholesterol level, and lymphocyte count but does not include body composition measures such as BMI (19). Because assessment of muscle mass is critical in considering the diagnosis of sarcopenia, the diagnostic power of CONUT can be low for detecting sarcopenia. The cut-off values of GNRI were rather different in the subclasses of T2DM participants. The GNRI cut-off values were associated with low SMI and diagnosis of sarcopenia in men and women (105), and BMI ≥ 22 (112) subclasses. While the GNRI cut-off value of 102 was associated with low handgrip strength and diagnosis of sarcopenia in patients with BMI < 22. When using an originally reported low nutrition-related cut-off of 98 (15), GNRI was associated with sarcopenia in men and women subclasses but not in the BMI < 22 and BMI ≥ 22 subclasses. The cut-off values of CONUT were not associated with a diagnosis of sarcopenia and its components in these subclasses, except in the diagnosis of sarcopenia in BMI < 22. The individual components of the CONUT score are shown in Supplementary Table 1. The distribution of scoring of lymphocyte count, total cholesterol, and albumin was not different between the sarcopenia– and sarcopenia+ groups. As discussed above, if the status of weight in the formula is applied, GNRI could be a better predictor for sarcopenia in the subclasses BMI < 22 and BMI ≥ 22.

TABLE 5 Multivariate logistic regression analysis (Model 2 in Table 4) on associations of cutoff of nutritional indices with diagnosis of sarcopenia.

	Independent variable							
	Albumin		GNRI		GNRI		CONUT	
Dependent variable	Cutoff: 4.20	*P*	Cutoff: 98	*P*	Cutoff: 105	*P*	Cutoff: 2	*P*
Men								
Low SMI	1.51 (0.79–2.90)	0.214	3.82 (1.52–9.57)	0.004	9.84 (4.73–20.47)	<0.001	1.10 (0.55–2.19)	0.796
Low hand grip strength	1.86 (0.81–4.29)	0.143	2.69 (0.99–7.31)	0.053	2.48 (1.10–5.59)	0.028	1.18 (0.51–2.75)	0.699
Low walking speed	0.36 (0.07–1.97)	0.236	1.54 (0.23–10.19)	0.656	0.98 (1.18–5.23)	0.983	0.94 (0.17–5.16)	0.940
Sarcopenia	2.15 (0.76–6.13)	0.151	3.76 (1.23–11.55)	0.021	5.29 (1.90–14.68)	<0.001	0.90 (0.32–2.56)	0.843
	Independent variable							
	Albumin		GNRI		GNRI		CONUT	
Dependent variable	Cutoff: 4.00	*P*	Cutoff: 98	*P*	Cutoff: 105	*P*	Cutoff: 1	*P*
Women								
Low SMI	2.15 (0.93–4.95)	0.073	5.29 (1.67–16.78)	0.005	12.04 (4.98–29.14)	<0.001	1.30 (0.52–3.26)	0.575
Low hand grip strength	5.42 (2.30–12.77)	<0.001	2.35 (0.73–7.53)	0.152	1.44 (0.64–3.24)	0.379	1.22 (0.52–2.87)	0.645
Low walking speed	2.99 (0.84–10.67)	0.093	0.84 (0.09–7.74)	0.877	0.44 (0.09–2.23)	0.321	0.71 (0.19–2.64)	0.608
Sarcopenia	7.69 (2.21–26.67)	<0.001	7.07 (1.75–28.58)	0.006	7.71 (2.24–26.54)	<0.001	0.58 (0.16–2.09)	0.407
	Independent variable							
	Albumin		GNRI		GNRI		CONUT	
Dependent variable	Cutoff: 3.70	*P*	Cutoff: 98	*P*	Cutoff: 102	*P*	Cutoff: 1	*P*
BMI < 22								
Low SMI	0.22 (0.06–0.77)	0.018	0.95 (0.41–2.21)	0.907	1.11 (0.50-2.46)	0.802	0.97 (0.37–2.56)	0.958
Low hand grip strength	2.22 (0.59–8.35)	0.240	3.51 (1.25–9.86)	0.017	5.32 (1.61–17.56)	0.006	0.55 (0.17–1.83)	0.333
Low walking speed	9.42 (0.57–154.68)	0.116	5.96 (0.46–76.65)	0.171	–		0.24 (0.01–7.28)	0.415
Sarcopenia	0.86 (0.18–4.10)	0.848	3.01 (0.10–9.08)	0.051	7.73 (1.77–33.78)	0.007	0.23 (0.05–0.97)	0.045

(Continued)

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TABLE 5 (Continued)

Dependent variable	Independent variable: Albumin Cutoff: 4.00	*P*	GNRI Cutoff: 98	*P*	GNRI Cutoff: 112	*P*	CONUT Cutoff: 2	*P*
BMI ≥ 22								
Low SMI	1.18 (0.51–2.72)	0.736	3.49 (0.29–42.05)	0.326	11.69 (3.34–40.50)	<0.001	0.74 (0.32–1.73)	0.485
Low hand grip strength	3.44 (1.68–7.08)	<0.001	6.59 (0.62–70.29)	0.119	1.53 (0.74–3.16)	0.250	1.60 (0.79–3.24)	0.189
Low walking speed	1.80 (0.57–5.65)	0.317	–		0.35 (0.11–1.13)	0.079	1.14 (0.38–3.44)	0.817
Sarcopenia	2.56 (0.89–7.36)	0.081	12.34 (0.72–212.73)	0.084	7.15 (1.45–35.33)	0.016	1.09 (0.37–3.22)	0.873
Dependent variable	**Independent variable: Albumin Cutoff: 4.4**	***P***	**GNRI Cutoff: 98**	***P***	**GNRI Cutoff: 105**	***P***	**CONUT Cutoff: 1**	***P***
Diabetes duration < 5 years								
Low SMI	0.08 (0.00–1.63)	0.100	–		14.10 (0.93–214.6)	0.057	34.1 (0.17–7,032.3)	0.195
Low hand grip strength	0.19 (0.01–3.61)	0.269	12.46 (0.27–582.9)	0.199	–		–	
Low walking speed	–		–		–		–	
Sarcopenia	0.02 (0.00–2.65)	0.113	–		67.23 (0.26–17,568.6)	0.138	–	
Dependent variable	**Independent variable: Albumin Cutoff: 4.00**	***P***	**GNRI Cutoff: 98**	***P***	**GNRI Cutoff: 105**	***P***	**CONUT Cutoff: 3**	***P***
Diabetes duration ≥ 5 years								
Low SMI	0.90 (0.48–1.70)	0.752	4.73 (2.27–9.83)	<0.001	9.39 (5.31–16.61)	<0.001	1.67 (0.80–3.46)	0.171
Low hand grip strength	2.89 (1.55–5.39)	<0.001	2.31 (1.07–4.96)	0.032	1.58 (0.87–2.85)	0.131	1.46 (0.66–3.23)	0.350
Low walking speed	1.97 (0.72–5.41)	0.186	1.39 (0.36–5.40)	0.635	0.68 (0.22–2.10)	0.506	1.39 (0.39–4.91)	0.612
Sarcopenia	2.71 (1.23–5.98)	0.014	5.20 (2.15–12.60)	<0.001	5.55 (2.51–12.30)	<0.001	1.76 (0.63–4.94)	0.280

Data are odds ratio (95% confidential intervals) as Model 2 in Table 4 corrected for age (years), sex, diabetes duration (years), HbA1c (%), and eGFR (ml/min/1.73 m^2).
GNRI, geriatric nutritional risk index; CONUT, controlling nutritional status; P, provability; SMI, skeletal mass index; BMI, skeletal mass index.

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5.3. Limitations

Our study had some limitations. First, because this study was conducted at a single university hospital, there may be a bias toward patients with a high risk of developing sarcopenia. Second, the number of patients and duration of observation might have been insufficient to assess the development of sarcopenia. Third, this was a cross-sectional observational study, and low albumin followed by lower GNRI and the prevalence of low SMI and sarcopenia are mutually well-correlated. Therefore, we could not determine a cause-and-result relationship. Further large-scale longitudinal studies are needed to corroborate the results of this study.

6. Conclusion

Results indicated that GNRI shows a superior diagnostic power in the diagnosis of sarcopenia. Additionally, its optimal cut-off points were useful as compared to a GNRI cut-off of 98, which is a commonly used diagnostic level for undernutrition. Future large and prospective studies will be required to confirm the utility of the cut-off for undernutrition individuals at risk for sarcopenia.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by the Fukushima Medical University Ethics Committee. The patients/participants provided their written informed consent to participate in this study.

Author contributions

KS and MSh contributed to the concept and design of the study and analyzed the data. HT, YT, MY, MSa, HS, and MSh participated in data collection. KS and MSh wrote the first draft with input from HT, YT, MY, MSa, HS, KT, HM, and JJK. All authors contributed to the discussion and approved the final manuscript.

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Conflict of interest

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.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2023.1087471/full#supplementary-material

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Differential metabolites in cirrhotic patients with hepatitis B and muscle mass loss

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Background: Sarcopenia leads to complications (infections, hepatic encephalopathy and ascites) and poor overall survival in patients with cirrhosis, in which the phenotypic presentation is loss of muscle mass. This study aimed to reveal the metabolic profile and identify potential biomarkers in cirrhotic patients with hepatitis B virus and muscle mass loss.

Method: Twenty decompensated cirrhotic patients with HBV and muscle mass loss were designated Group S; 20 decompensated cirrhotic patients with HBV and normal muscle mass were designated Group NS; and 20 healthy people were designated Group H. Muscle mass loss was defined as the skeletal muscle mass index less than 46.96cm^2/m^2 for males and less than 32.46cm^2/m^2 for females. Gas chromatography–mass spectrometry was used to explore the distinct metabolites and pathways in the three groups.

Results: Thirty-seven metabolic products and 25 associated metabolic pathways were significantly different in the Group S patients from Group NS patients. Strong predictive value of 11 metabolites (inosine-5′-monophosphate, phosphoglycolic acid, D-fructose-6-phosphate, N-acetylglutamate, pyrophosphate, trehalose-6-phosphate, fumaric acid, citrulline, creatinine, (r)-3-hydroxybutyric acid, and 2-ketobutyric acid) were selected as potential biomarkers in Group S patients compared with Group NS patients. Two pathways may be associated with loss of muscle mass in patients with liver cirrhosis: amino acid metabolism and central carbon metabolism in cancer.

Conclusion: Seventy differential metabolites were identified in patients who have liver cirrhosis and loss of muscle mass compared with patients who have cirrhosis and normal muscle mass. Certain biomarkers might distinguish between muscle mass loss and normal muscle mass in HBV-related cirrhosis patients.

KEYWORDS

hepatitis B virus-related liver cirrhosis, muscle mass loss, sarcopenia, differential metabolites, amino acid metabolism, gut-liver-muscle axis

Introduction

Sarcopenia is a progressive and diffuse loss of muscle mass, strength, and function. Sarcopenia can be classified into age-related sarcopenia and secondary sarcopenia, the latter being caused by chronic diseases (1). The prevalence of sarcopenia in patients liver cirrhosis is about 40–70% (2). A consensus definition for sarcopenia in these patients is loss of muscle mass (3, 4), which is associated with poor

prognosis, including reduced quality of life, increased risk of infection, prolonged hospitalization, and increased rate of mortality (5, 6). Previous studies have focused on the pathology of sarcopenia and its associated factors. In patients with cirrhosis, abnormalities of the gut-liver-axis may be associated with the development of sarcopenia (4, 7). Cirrhosis itself leads to skeletal muscle disorders through several pathways: altered catabolic state, altered protein metabolism, and impaired hepatic ammonia clearance (8), and the metabolic disorders may be the root cause of the low muscle mass. Metabolomics, an emerging discipline, is frequently used to investigate pathophysiological processes involved in disease progression and the identification of new diagnostic or prognostic biomarkers (9, 10). Patients with alcoholic liver disease and cirrhosis have alterations in metabolites, as in nucleic acids and amino acids (11). Patients with non-alcoholic steatohepatitis (NASH)-related cirrhosis have an increased risk of sarcopenia because of the additive effects of insulin resistance and chronic systemic inflammation (12). Cholestasis-predominant liver diseases, such as primary sclerosing cholangitis, have elevated serum bile acid levels, which may induce skeletal muscle atrophy through the bile acid receptor, TGR5, that is expressed in healthy muscles (13, 14). However, the differential metabolites in hepatitis B virus (HBV)-related decompensated liver cirrhosis with loss of muscle mass vs. normal muscle mass are mostly unknown. Therefore, our study aimed to characterize the metabolic profile and identify potential biomarkers of muscle mass loss in HBV-related decompensated liver cirrhosis.

Materials and methods

Study population

Patients with HBV-related liver cirrhosis were recruited between August 2021 and June 2022 at the Affiliated Hospital of Qingdao University. All participants provided written informed consent. The diagnosis of cirrhosis was made on the combination of clinical and laboratory features or by liver histopathology (15, 16). Decompensated cirrhosis was defined as patients symptomatic with ascites or gastrointestinal bleeding, or so on (17, 18). Patients were excluded for (1) causes of cirrhosis other than chronic HBV infection, such as alcoholic cirrhosis, autoimmune cirrhosis, and others, (2) age over 65 years, (3) acute-chronic and acute liver failure, (4) other diseases that can lead to secondary muscle depletion, such as chronic diseases of the heart, lungs, kidneys, or brain, and malignant tumors (5) neurodegenerative or muscle degenerative diseases, (6) perioperative patients, (7) patients without computed tomography (CT) scan within 3 months, (8) the Nutrition Risk Screening 2002 score of the patients was over 3 points (19), and (9) patients who have exercise habits with Physical Activity Rating Scale-3 > 19 points (20). Patients excluded from the healthy control group were those with metabolic diseases (diabetes, thyroid disorder, and others) and other chronic diseases, according to their ultrasound and laboratory assessments.

Skeletal muscle measurement and diagnostic criteria

Skeletal muscle mass index (SMI) is defined as the ratio of lean tissue area to body height. Lean tissue area is the skeletal muscle area (SMA) calculated according to CT readings at the level of the third lumbar vertebra (L3). Muscle attenuation (MA), which is associated with muscle density and intramuscular lipid content, is the mean Hounsfield unit (HU) of the entire SMA (21). All the recruited patients had abdominal CT scans at admission. Two sequential transverse CT images at the level of L3 were analyzed with SliceOmatic V5.0 software (Tomovision, Montreal, QB, Canada), which enables specific tissue demarcation using HU thresholds. The CT HU thresholds were −29 to 150 for quantifying muscle mass; −150 to −50 for visceral adipose tissue; and −190 to −30 for subcutaneous fat tissue. The formula for SMI was SMI (cm^2/m^2) = SMA (cm^2)/height (m^2). Muscle mass loss was defined as SMI less than 46.96 cm^2/m^2 for males and less than 32.46 cm^2/m^2 for females (22).

Clinical and laboratory assessments

Morning fasting blood was collected and centrifuged at 3000×g for 15 min. The plasma was stored at −80°C until the gas chromatography–mass spectrometry (GC–MS) analysis was conducted.

Preparation of samples

Samples were thawed at room temperature before analysis. One-hundred-and-fifty microliters of samples were placed in a 1.5-mL Eppendorf tube with L-2-chlorophenylalanine (0.06 mg/ml) dissolved in methanol as internal standard, and the tube was vortexed for 10 s. An ice-cold mixture of methanol and acetonitrile (vol:vol, 2:1) was added, and the mixtures were vortexed for 30 s. The whole samples were extracted by ultrasound for 10 min in ice-water bath and placed at −20°C for 30 min. The extract was centrifuged at 4°C (13,000 rpm) for 10 min. 150 µl of supernatant in a glass vial were dried in a freeze-concentration centrifugal dryer, and 80 µl of 15 mg/ml methoxyamine hydrochloride in pyridine was added. The mixture was vortexed vigorously for 2 min and incubated at 37°C for 90 min. 50 µl of bistrifluoroacetamide with 1% trimethylsilyl chloride and 20 µl n-hexane were added into the mixture, which was vortexed vigorously for 2 min and then derivatized at 70°C for 60 min. The samples were placed at ambient temperature for 30 min before GC–MS analysis.

Gas chromatography–mass spectrometry analysis

The derivatized samples were analyzed on an Agilent 7890B gas chromatography system coupled to an Agilent 5977A MSD system (Agilent Technologies Inc., Santa Clara, CA, United States). A DB-5MS fused-silica capillary column (30 m × 0.25 mm × 0.25 µm) (Agilent J & W Scientific, Folsom, CA, USA) was used to separate the derivatives. Helium (>99.999%) was used as the carrier gas at a constant flow rate of 1 ml/min through the column. The injector temperature was maintained at 260°C. The initial oven temperature was 60°C, held at 60°C for 0.5 min, ramped to 125°C at a rate of 8°C/min, to 210°C at a rate of 5°C/min, to 270°C at a rate of 10°C/min, to 305°C at a rate of 20°C/min, and finally held at 305°C for 5 min. The temperature of MS quadrupole and ion source (electron impact) were set to 150°C and 230°C, respectively. The collision energy was 70 eV. Mass spectrometric data were acquired in a full-scan mode (m/z 50–500), and the solvent delay time was set to 5 min. The quality control samples (QC) were injected at regular intervals (every 10 samples) throughout the analytical run to provide a set of data from which repeatability could be assessed.

TABLE 1 Demographic and clinical characteristics of the study participants.

	Cirrhosis with muscle mass loss (Group S) *N*=20	Cirrhosis with normal muscle mass (Group NS) *N*=20	Healthy (Group H) *N*=20	Value of *p*
Age	48 (29–62)	49.8 (32–61)	31 (22–42)	**<0.05**
Gender (*n*) Male/Female	16/4	14/6	9/11	
BMI	23.17 (21.84–25.92)	25.63 (22.54–28.10)	23.45 (20.85–24.75)	0.06
Child-Pugh stage (*n*)				
A	8	6		
B	9	6		
C	5	8		
MELD score	12.10 ± 3.42	12.55 ± 4.07		0.07
SMI	40.29 ± 4.85	50.32 ± 6.60		**<0.05**
SMA	121.06 ± 18.02	144.71 ± 27.45		**<0.05**
MA	39.81 ± 5.45	43.17 ± 5.20		0.053
SFA	99.29 ± 55.24	133.36 ± 64.75		0.081
VFA	83.13 ± 55.53	78.21 ± 55.17		0.780
Pre-albumin	75.59 ± 34.35	96.7 ± 58.69		0.174
Total protein	59.43 ± 8.83	60.97 ± 10.40		0.618
Albumin	31.60 ± 5.94	31.14 ± 6.63		0.817
Albumin/ Globulin	1.13 ± 0.26	1.07 ± 0.21		0.448
ALT	19 (14–29.75)	29.5 (24.5–55)		**<0.05**
AST	27 (19.75–41.75)	34 (28–60.5)		0.053
GGT	16.5 (12.25–40.75)	30.5 (19.75–68.03)		0.064
ALP	76.5 (51–89)	101 (70–142)		**<0.05**
Total bilirubin	29.47 ± 17.08	40.76 ± 27.83		0.132
Direct bilirubin	9.05 (6.68–11.13)	10.55 (5.53–27.95)		0.358
Creatinine	78.45 (72.5–89)	74.85 (68.25–95.25)		0.482
BUN	5.17 (3.90–7.52)	4.58 (3.67–6.10)		0.552
D-dimer	855 (322.5–1765)	525 (247.5–962.5)		0.133
PT	16.66 ± 2.52	15.76 ± 2.76		0.291
INR	1.41 ± 0.23	1.35 ± 0.24		0.421
Fibrinogen	2.00 ± 0.52	1.85 ± 0.52		0.432

BMI, body mass index; SMI, skeletal muscle mass index; SMA, skeletal muscle area; MA, muscle attenuation; VFA, visceral adipose tissue; SFA, subcutaneous fat tissue; ALT, alanine transaminase; AST, aspartate aminotransferases; GGT, gamma glutamyl transferase; ALP, alkaline phosphatase; BUN, blood urea nitrogen; PT, prothrombin time; INR, International Normalized Ratio. The bold values means: *p* value <0.05 indicated statistically significant differences.

Statistical analysis

SPSS 26.0 software (International Business Machines Corp., Chicago, IL, United States) was used for the statistical analyses. Mean ± standard deviation was used in quantitative variables, and the significance was determined with a Student's t-test. Non-normally distributed variables are expressed as a median and interquartile range, and the significance was determined using a Mann–Whitney U test. *p* value <0.05 indicated statistically significant differences. A systematic analysis of the metabolites was identified and confirmed *via* searching the Kyoto Encyclopedia of Genes and Genomes (KEGG) database.[1]

1 https://www.kegg.jp

Ethics statement

The study protocol was approved by the ethics board of the affiliated hospital of Qingdao University (No. QYFYWZLL26461). We also registered this study on ClinicalTrials.gov (NCT05041348).

Results

Baseline clinical characteristics of patients

The baseline clinical characteristics of the patients with HBV-related liver cirrhosis are summarized in Table 1. Twenty decompensated cirrhotic patients with HBV and muscle mass loss (Group S), 20 decompensated cirrhotic patients with HBV and normal muscle mass (Group NS), and 20 healthy people (Group H) were included in this

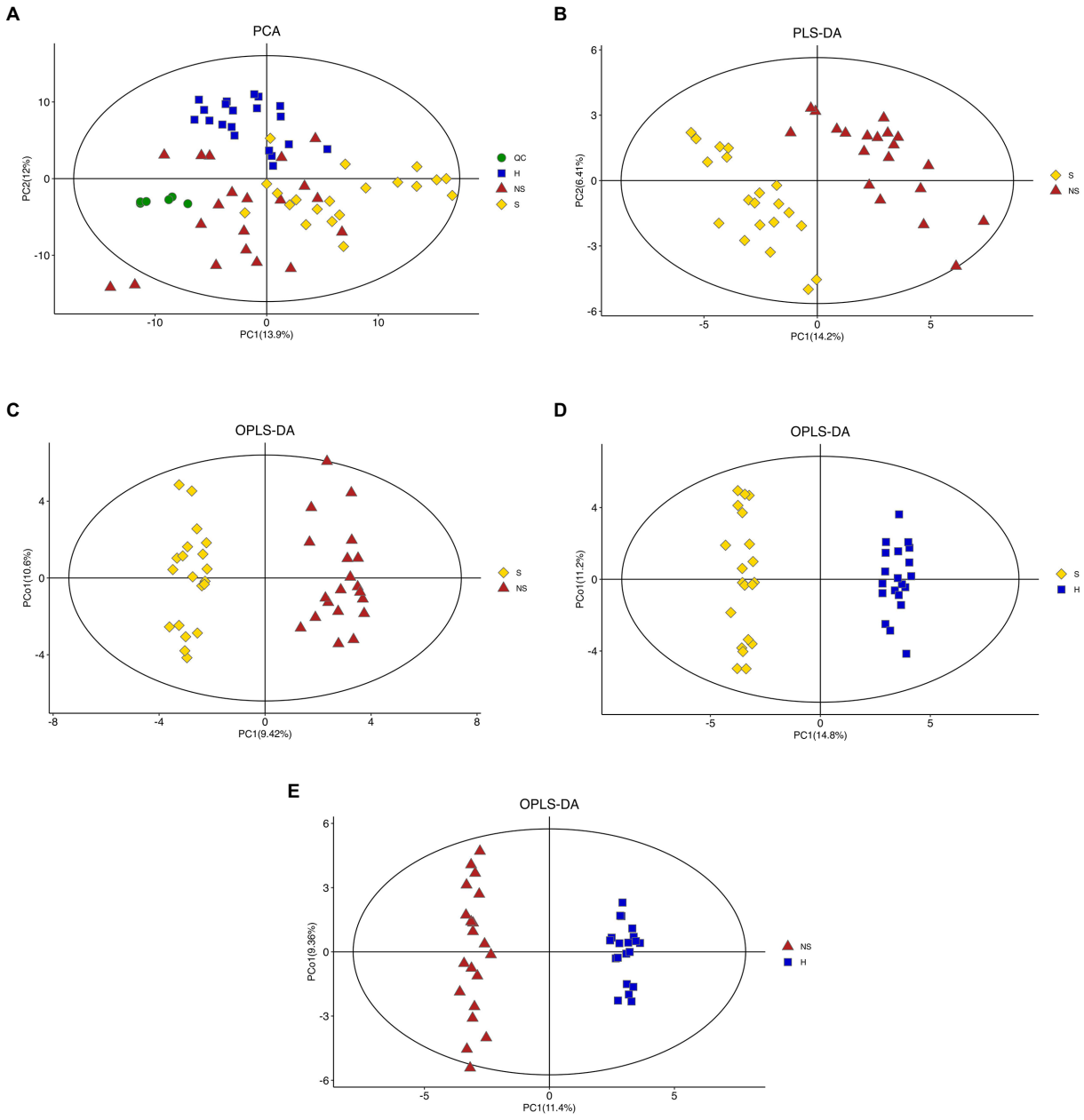

FIGURE 1
Score plot. **(A)** Unsupervised principal component analysis (PCA) plots of all groups (QC, Quality control samples; H, Health control group; S, HBV-related decompensated liver cirrhosis patients with muscle mass loss; NS, liver cirrhosis patients with normal muscle mass), **(B,C)** Partial least-squares-discriminant analysis (PLS-DA) and orthogonal partial least-squares discriminant analysis (OPLS-DA) score plots of Group S and Group NS, **(D)** OPLS-DA score plots of Group S and Group H, and **(E)** OPLS-DA score plots of Group NS and Group H.

study. There were no significant differences in BMI and the model for end-stage liver disease score (MELD score) among the groups ($p > 0.05$). The SMI and SMA in Group S were significantly lower than in Group NS ($p < 0.05$). Alanine transaminase and alkaline phosphatase values were significantly higher in Group NS than in Group S ($p < 0.05$).

Overall metabolomics analysis of samples

The unsupervised principal component analysis (PCA) revealed that the healthy control group (H) could be distinguished from other groups (Figure 1A). The QC samples clustered tightly, indicating stability of the method. PCA was used to reveal the overall distribution among the samples and the stability of the whole analysis. To exclude possible confounding variables, partial least-squares-discriminant analysis (PLS-DA) and orthogonal partial least-squares discriminant analysis (OPLS-DA) were used to distinguish the metabolites that differed between groups (Figures 1B,C). Although the distribution of Group S samples overlapped with the distribution of Group NS samples, the PLS-DA and OPLS-DA (R2Y = 0.958, Q2 = 0.683, and R2Y = 0.958, Q2 = 0.715, respectively) indicated high cross-validation predictability and goodness-of-fit. The OPLS-DA also revealed significant discrimination between Group S and Group H (Figure 1D, R2Y = 0.988 and Q2 = 0.897) and between Group NS and Group H (Figure 1E,

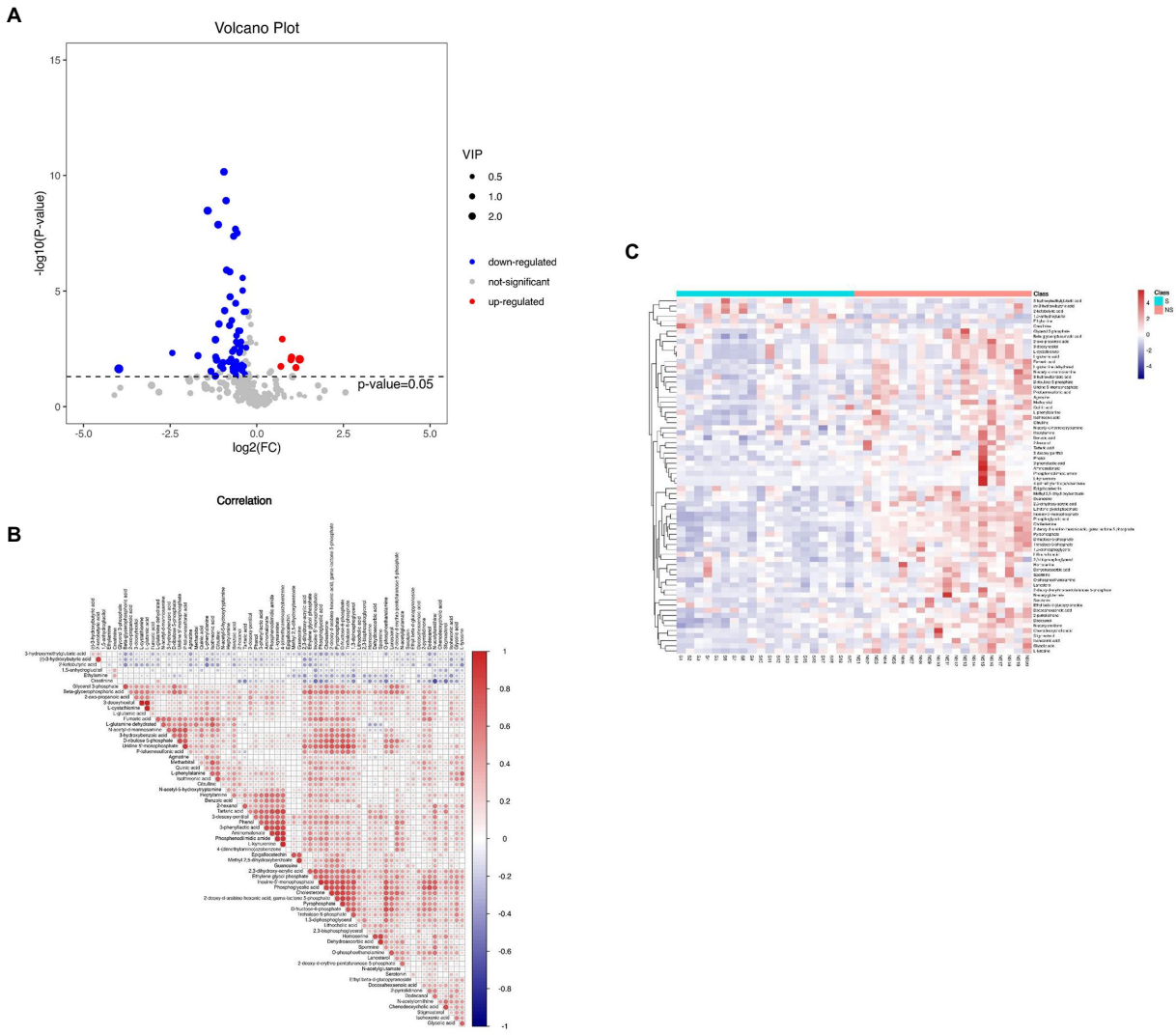

FIGURE 2
(A) The volcano plot of Groups S and NS, **(B)** Correlation analysis of metabolites in Group S and NS, and **(C)** The heatmap of the correlation analysis revealed the expression level of metabolites in Groups S and NS.

R2Y = 0.99 and Q2 = 0.834.). The combined results indicated reliable differences among the three groups.

Metabolites profiling in the three groups

A total of 162 volatile metabolites were identified in the three groups, with variable importance on projection (VIP) scores >1 and p values <0.05. With Group S and Group H combined, 102 differential metabolites were detected. Ninety-three metabolites were found in Group NS and Group H. Information of the metabolites is illustrated in Supplemental Figure 1. To identify potential biomarkers that may be related to muscle mass loss, we compared the metabolite profile between Group S and Group NS. Seventy differential metabolites (consisting mainly of organic acids and derivatives, lipids, and lipid-like molecules, and organic oxygen compounds) were detected with VIP >1 and $p < 0.05$. The visualized volcano plot (Figure 2A) showed the correlation of the metabolites between Group S and Group NS, including elevated levels of ethylamine, (r)-3-hydroxybutyric acid, 3-hydroxymethylglutaric acid, 2-ketobutyric acid, 1,5-anhydroglucitol and creatinine. The levels of 64 metabolites were downregulated in Group S. Correlation analysis illustrated the relationships among the metabolites (Figure 2B). A heatmap of the correlation analysis revealed the expression levels of the metabolites (Figure 2C).

Pathways related to liver cirrhosis with muscle mass loss

Twenty-five pathways were defined as disturbed in the plasma of liver cirrhosis patients who had muscle mass loss (10 pathways with p values <0.01 and 15 pathways with p value <0.05). Thirty-seven differential metabolites were enriched in the disturbed pathways; the corresponding KEGG map of these potential biomarkers, the value of fold change, and other information are presented in Supplemental Table 1. Most of the disturbed pathways were related to amino acid metabolism (Figure 3). Node color is based on the p value (red indicates a higher level of significance), and node radius is determined by the number of differential metabolites in this pathway. The relationships between metabolites and pathways are illustrated in Supplemental Table 2.

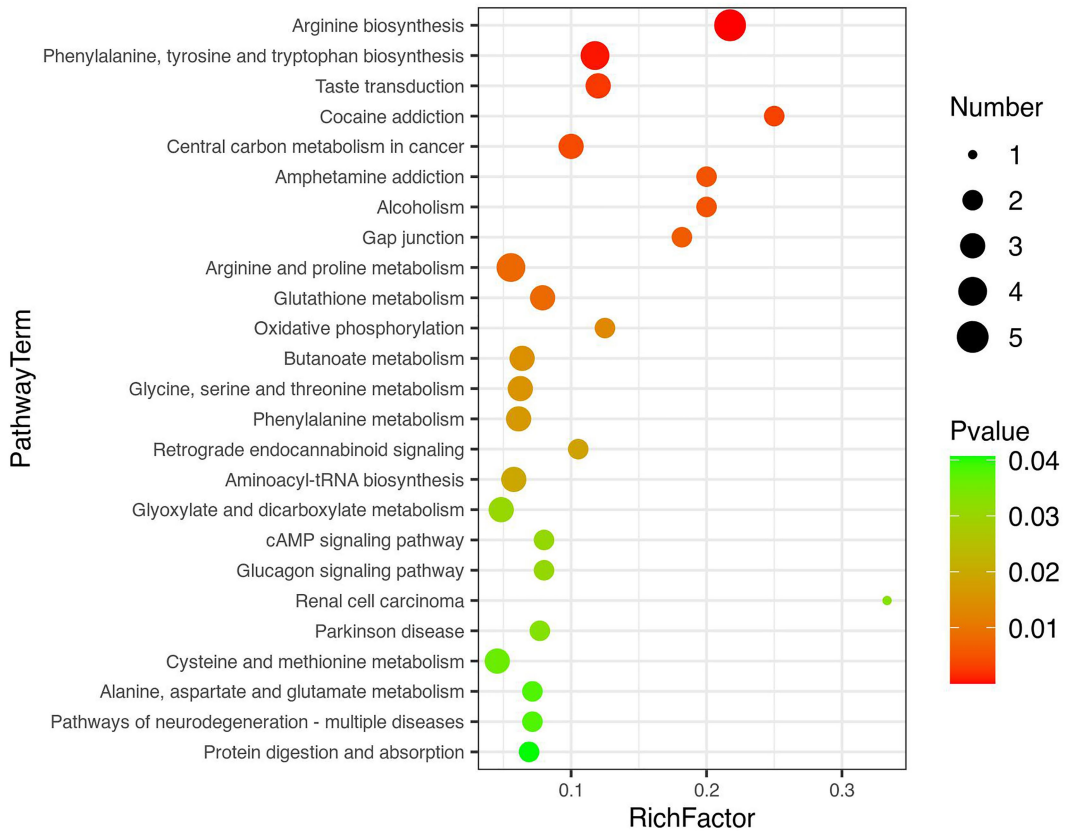

FIGURE 3
Pathway enrichment and topology analysis.

Differential metabolites detected in group S and NS

Among the differential metabolites in the groups, three differential metabolites with enrichment pathways appeared in Group S compared with Group NS. Citrulline (VIP = 1.59, $p = 0.003$) and agmatine (VIP = 1.02, $p = 0.039$) were downregulated in Group S, and 2-ketobutyric acid (VIP = 1.73, $p = 0.020$) was upregulated in Group S. Creatinine (VIP = 1.473, $p = 0.001$) and (r)-3-hydroxybutyric acid (VIP = 2.02, $p = 0.007$) levels were elevated in Group S but not as unique metabolites. Inosine-5′-monophosphate (VIP = 2.24, $p < 0.001$), with the highest VIP score, was decreased in Group S. L-glutamic acid (VIP = 1.45, $p = 0.028$) and L-tyrosine (VIP = 1.16, $p = 0.011$), which appeared in almost all the enrichment pathways, may be the hub of various metabolic pathways related with muscle mass loss. All the results suggested that amino acid metabolism and central carbon metabolism in cancer are associated with muscle mass loss in patients with liver cirrhosis.

Potential biomarkers selected by receiver operator characteristic curve

To further identify the key metabolites that could distinguish liver cirrhosis with muscle mass loss from those with normal muscle mass, ROC curves were analyzed (Figures 4A,B). The area under the curve (AUC) was used to assess the potential diagnostic value: inosine-5′-monophosphate, 0.9825 (95% confidence interval (CI) 0.946–1.000), phosphoglycolic acid, 0.975 (95% CI 0.937–1.000), D-fructose-6-phosphate, 0.95 (95% CI 0.887–1.000); N-acetylglutamate, 0.9125 (95% CI 0.829–0.996); pyrophosphate, 0.905 (95% CI 0.806–0.988); trehalose-6-phosphate, 0.87 (95% CI 0.752–1.000); fumaric acid, 0.82 (95% CI 0.687–0.953); citrulline, 0.7725 (95% CI 0.627–0.918); creatinine, 0.815 (95% CI 0.684–0.946); (r)-3-hydroxybutyric acid, 0.775 (95% CI 0.618–0.932); 2-ketobutyric acid, 0.7275 (95% CI 0.559–0.896). The AUCs were >0.7, indicating the diagnostic accuracy of these metabolites.

Discussion

In this study, we identified by GC–MS analysis the differential metabolites in cirrhotic patients with HBV and muscle mass loss. The results revealed clear separation of three groups (decompensated liver cirrhosis with muscle mass loss; decompensated liver cirrhosis with normal muscle mass; and healthy people). To the best of our knowledge, this is the first study that analyzed the plasma metabolic characteristics of patients with HBV-related liver cirrhosis and associated loss of muscle mass. We found that there were two pathways that may be associated with loss of muscle mass in patients with liver cirrhosis: amino acid metabolism, and central carbon metabolism in cancer. Moreover, inosine-5′-monophosphate, phosphoglycolic acid, D-fructose-6-phosphate, N-acetylglutamate, pyrophosphate, trehalose-6-phosphate, fumaric acid, citrulline, creatinine, (r)-3-hydroxybutyric acid, and 2-ketobutyric acid were found potential biomarkers in HBV-related liver cirrhosis with muscle mass loss.

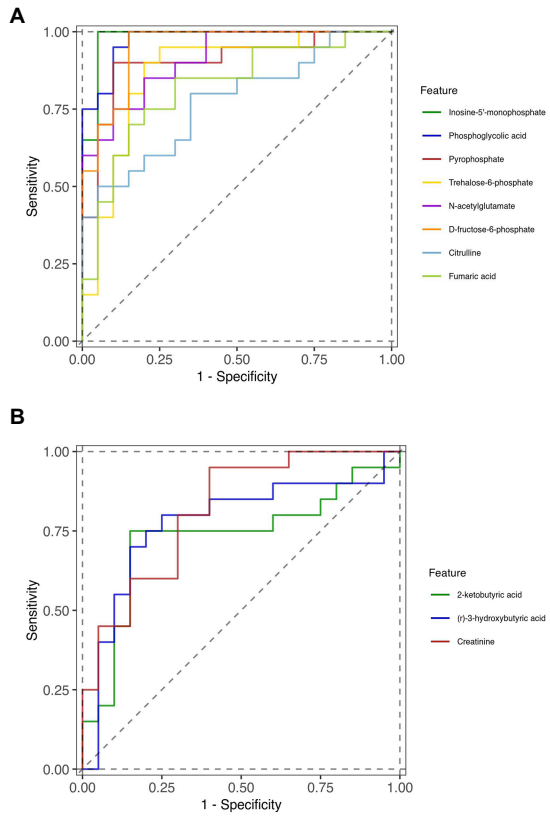

FIGURE 4
ROC curves of the diagnostic metabolites. **(A)** downregulated metabolites in Group S and **(B)** upregulated metabolites in Group S.

Arginine is a semi-essential amino acid that participates in the urea cycle, the nitric oxide (NO) cycle, and muscle protein synthesis. Citrulline, as a non-essential amino acid, can be metabolized to arginine in the urea and NO cycles and can control the delivery of arginine to the liver. However, higher levels of circulating arginine induce arginases, which result in rapid arginine clearance. Therefore, citrulline is arginine's precursor (23, 24). Increased levels of citrullines may be crucial in promoting muscle function (25). Bailey et al. (26) found that citrulline, an effective dietary supplement, is better than arginine in improving oxidative metabolism and exercise performance. Caballero-Garcia et al. (27) reported that muscle strength and endurance tended to increase with citrulline supplementation. In this study, citrulline, as a differential metabolite, was specifically downregulated in liver cirrhosis patients who had muscle mass loss. The corresponding arginine biosynthesis pathway was also found an enrichment pathway with a lower p value ($p < 0.001$). These results are consistent with previous research and confirmed that decreased plasma concentrations of citrulline are related to muscle mass loss. More studies to investigate the relationship between citrulline and muscle mass loss in patients with liver cirrhosis are needed.

(R)-3-hydroxybutyric acid, one of the ketone bodies in humans, is produced by the liver during carbohydrate deprivation to provide energy to brain, heart, and muscle cells (26). In the fasting state or with decreased oral intake, ketone bodies are increased to provide energy and regulate lipolysis, proteolysis, and ketogenesis (27–29). Studies of ketone body supplementation in athletes and healthy people found that the supplementation increases metabolic flexibility during exercise and enhances endurance exercise performance (30–32). However, we found that (r)-3-hydroxybutyric acid (VIP >2, and FC >2) were increased in liver cirrhosis patients with muscle mass loss. ROC analysis had an AUC of 0.775, implying high specificity and sensitivity. Liver cirrhosis disturbs energy synthesis and metabolism, which in turn influences the nutritional status (33). In disease states, the utilization efficiency of ketone bodies may decrease. Sasaki et al. (32) found that high concentrations of venous ketone bodies in hepatocellular carcinoma patients were related with low skeletal muscle quality. The relationship between ketone bodies, liver cirrhosis, and muscle mass loss is still ambiguous. Our results provide directions for future research.

Creatinine (VIP =1.47, $p < 0.05$, and AUC = 0.815), detected as a biomarker, was increased in Group S. It is a breakdown product of creatine phosphate in muscle. Hence, under the state of stable renal function and normal nutrition intake condition, creatinine concentration can reflect muscle mass (34, 35). Some researchers combined creatinine and cystatin C as a novel index to evaluate muscle mass and a low serum creatinine level, most likely due to sarcopenia, is associated with a higher mortality rate (36, 37). In our study, we excluded patients with kidney disease that can lead to secondary muscle depletion. The method in our hospital to detect creatinine concentration is the Picric acid method which is different from GC–MS. The concentration of creatinine (μmol/L) in the cirrhotic group with and without loss of muscle mass was 78.45 (72.5–89) and 74.85 (68.25–95.25) respectively. It was consistent with the results of GC–MS. According to our results, creatinine may as a sensitive indicator to predict muscle mass loss.

Inosine-5′-monophosphate (IMP), which participates in purine metabolism, is generated by a *de novo* biosynthetic pathway in the liver (38). IMP can be converted to adenosine diphosphate or guanosine-5′-diphosphate and then to adenosine triphosphate or guanosine trisphosphate (39). IMP is an important indicator of meat quality and freshness in animals (40, 41). However, the role of IMP in humans is rarely mentioned. One study concluded that IMP is in low amount in human skeletal muscle at rest and after low-intensity exercise, but it is formed after moderate and high-intensity exercise (42). We found that IMP was downregulated in patients with muscle mass loss. In the future, IMP, which can increase energy availability and protein synthesis, may be considered for use as a dietary supplement.

In this study, phosphoglycolic acid (VIP >2, $p < 0.05$, and AUC = 0.975), a differential metabolite, was decreased in patients with liver cirrhosis and muscle mass loss. Phosphoglycolic acid can be regulated by phosphoglycolate phosphatase, which is a recently identified mammalian enzyme at the intersection of glucose metabolism, lipogenesis, lipolysis, and cellular nutrient-excess detoxification (43, 44). The relationship between phosphoglycolate phosphatase activity, liver function, and muscle mass was not clear. More studies are needed to determine the function of phosphoglycolic acid in muscle metabolism.

Our study has strengths: First, sarcopenia can be classified into age-related sarcopenia and secondary sarcopenia. Despite advanced cirrhosis, we excluded patients over 65 years old and other diseases that can lead to secondary muscle depletion, such as chronic diseases of the heart, lungs, kidneys, or brain, malignant tumors, and neurodegenerative or muscle degenerative diseases. In our study, patients with a single cause of liver cirrhosis (HBV infection) were studied, which favored increased homogeneity of the study population. Second, our results identified differential metabolites that may lead to liver cirrhosis-related muscle mass loss and, thus, provided directions for future research.

However, the study has limitations: Although, before we started this study, we calculated the skeletal muscle mass index of 101 liver cirrhosis patients at our hospital. 80 patients can be defined as

having low muscle mass. The sample size that we calculated according to the prevalence (79.21%) of liver cirrhosis with low muscle mass at our hospital was around 10.22. And we recruited 20 patients from each group. The current sample size is sufficient to illustrate the conclusion of this manuscript. However, this is a single-center study with a small sample size, and the results will need testing on a larger series of patients. Second, we did not screen for branched chain amino acids as differential metabolites; the relationship of branched chain amino acids with sarcopenia seems clear from the results of recent research: some researchers have reported that branched amino acid treatment improves muscle mass of cirrhotic patients with sarcopenia (45). Finally, we only investigated muscle mass loss through imaging features, not muscle strength, and decreased muscle strength is another characteristic of sarcopenia. Thus, the differential expression of metabolites in liver cirrhosis should be a subject of future research.

In conclusion, this is the first analysis of the plasma metabolic characteristics of HBV-related decompensated liver cirrhosis with accompanying loss of muscle mass. Through GC–MS studies, 37 metabolites with 25 pathways were found disturbed in the plasma of patients with cirrhosis. The potential value of 11 metabolites (inosine-5′-monophosphate, phosphoglycolic acid, D-fructose-6-phosphate, N-acetylglutamate, pyrophosphate, trehalose-6-phosphate, fumaric acid, citrulline, creatinine, (r)-3-hydroxybutyric acid, and 2-ketobutyric acid) as biomarkers of muscle mass was identified. Two pathways may be associated with loss of muscle mass in patients with HBV-related decompensated liver cirrhosis, i.e., amino acid metabolism and central carbon metabolism in cancer.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author.

Ethics statement

The study protocol was approved by the ethics board of the affiliated hospital of Qingdao University (No. QYFYWZLL26461). We also registered this study on ClinicalTrials.gov (NCT05041348). The patients/participants provided their written informed consent to participate in this study.

Author contributions

XL and XJ: conceptualization. LH, GG, and QN: methodology. QS: software. SB and XL: formal analysis. XD: investigation. YJ and XJ: data curation and supervision. XL: writing—original draft preparation. XJ: writing—review and editing and project administration. All authors contributed to the article and approved the submitted version.

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Conflict of interest

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.

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2023.1068779/full#supplementary-material

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Intake of omega-3 polyunsaturated fatty acids and fish associated with prevalence of low lean mass and muscle mass among older women: Analysis of Korea National Health and Nutrition Examination Survey, 2008-2011

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The effects of dietary n-3 PUFA and fish on the risk of sarcopenia and muscle mass remain unclear. The present study investigated the hypothesis that intake of n-3 PUFA and fish is negatively associated with the prevalence of low lean mass (LLM) and positively correlated with muscle mass in older adults. Data from the Korea National Health and Nutrition Examination Survey, 2008-2011, 1,620 men and 2,192 women aged over 65 years were analyzed. LLM was defined as appendicular skeletal muscle mass divided by body mass index < 0.789 kg for men and <0.512 kg for women. Women and men with LLM consumed less eicosapentaenoic acid (EPA) docosahexaenoic acid (DHA) and fish. In women, but not men, the prevalence of LLM was associated with the intake of EPA and DHA (odds ratio, 0.65; 95% confidence interval, 0.48-0.90; $p = 0.002$) and fish (odds ratio, 0.59; 95% confidence interval, 0.42-0.82; $p < 0.001$). Muscle mass was also positively associated with the intake of EPA, DHA ($p = 0.026$), and fish ($p = 0.005$) in women, but not men. α-Linolenic acid intake was not associated with the prevalence of LLM and was not correlated with muscle mass. The findings suggest that consumption of EPA, DHA, and fish are negatively associated with the prevalence of LLM, and positively correlated with muscle mass in Korean older women, but not in older men.

KEYWORDS

low lean mass, muscle mass, sarcopenia, n-3 PUFA intake, fish intake, older women, KNHANES

1. Introduction

Sarcopenia, an age-associated loss of muscle mass and, strength, or performance is associated with increased adverse outcomes including falls, functional decline, frailty, and mortality, and has become a serious health issue among older adults (1). There are various complicated risk factors for sarcopenia, including aging, body composition,

physical activity,comorbidities, and dietary intake (1). Malnutrition is well-known risk factor for sarcopenia, but the effect of individual nutrient such as protein, vitamin D, and n-3 polyunsaturated fatty acids (PUFA) on sarcopenia is unclear (2, 3). N-3 PUFA, eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA), which are abundant in fish, and α-linolenic acid (ALA), which is abundant in plants, have anti-inflammatory effects (4). It is becoming increasingly clear that inflammation processes play an important role in the pathogenesis of age-related sarcopenia (4). Low lean mass is one of the first factors to diagnose sarcopenia (1).

The risk of sarcopenia is negatively associated with the intake of total n-3 PUFA in kidney transplant patients (5) and serum levels of total n-3 PUFA in Korean older adults (6). Similarly, the ratio of daily intake of total n-3 PUFA to energy intake has been significantly associated with the risk of sarcopenic obesity in Korean older women, but not men, suggesting that n-3 PUFA have beneficial effects only in women (7). In kidney transplant patients, the intake of total n-3 PUFA have also been positively associated with muscle mass and negatively associated with the risk of low muscle mass (5).

Consistent with muscle mass, intakes of total n-3 PUFA, EPA, and DHA were positively associated with muscle function in American older adults (8), Japanese men (9), Finnish women (10), and Korean women (11). Sarcopenic older adults consumed less total n-3 PUFA and had lower erythrocyte EPA levels in the Maastricht Sarcopenia Study (12). Plasma levels of total n-3 PUFA, EPA, and DHA, as indicators of dietary intake of n-3 PUFA, were positively associated with muscle function among older adults in Europe (12–14), America (15), and Korea (6, 16). In addition, a meta-analysis of clinical trials found that supplementation with EPA and DHA increased muscle mass and muscle performance measured by timed up-and-go and gait speed in older adults (17). n-3 PUFA have been suggested to have anabolic and anti-catabolic properties in skeletal muscles by regulating the mammalian target of rapamycin (mTOR) signaling pathway and inflammatory factors (18).

Dietary intake of fatty fish was positively associated with grip strength in older adults in the Hertfordshire cohort study (19) and UK Biobank study (20). Similarly, adherence to the Mediterranean diet, which is known to contain abundant n-3 PUFA, was associated with better physical performance in postmenopausal women (21) and a lower risk of sarcopenia in Iranian older adults (22).

ALA intake was positively associated with muscle function measured by gait speed, one-leg stance, squat, Short Physical Performance Battery (SPPB), and grip strength, but not with muscle mass in older women in Finland (10). Supplementation with ALA increased muscle mass but not muscle function in older men but not in women (23).

To our knowledge, no study has shown an association between dietary intake of EPA and DHA, ALA, and fish and the prevalence of sarcopenia and muscle mass in older adults. Therefore, the purpose of the present study was to investigate the hypothesis that consumption of n-3 PUFA and fish is negatively associated with the prevalence of LLM and positively correlated with muscle mass in older men and women.

2. Methods

2.1. Participants

This study was based on data obtained from the Korea National Health and Nutrition Examination Survey (KNHANES) from 2008 to 2011. KNHANES was performed using a rolling sampling design involving a complex, stratified, multistage, probability-cluster survey of a representative sample of the non-institutionalized civilian population in South Korea (24). The survey was performed by the Korean Ministry of Health and Welfare and consisted of three components: a health interview survey, health examination survey, and nutrition survey. All participants signed an informed consent form (24). The study protocol was approved by the Institutional Review Board of Hanyang University (HYUIRB-202208-003).

Of the 37,753 participants, 33,941 were excluded for the following reasons: age 65 years or younger (n = 31,383); missing data on body mass index (BMI, kg/m^2), appendicular skeletal muscle mass (ASM), and energy intake (n = 2,480); and extreme energy intake of less than 500 kcal/day or more than 4,000 kcal/day (n = 78). Finally, 1,620 men and 2,192 women were included in the present study (**Figure 1**).

2.2. Definition of low lean mass

Muscle mass was measured by dual-energy X-ray absorptiometry using a DISCOVERY-W fan-beam densitometer (Hologic, Marlborough, MA, USA). ASM (kg) was calculated as the sum of lean soft tissue in the bilateral upper and lower limbs. LLM was defined as $<$ 0.789 kg of ASM/BMI in men and $<$ 0.512 kg of ASM/BMI in women (25).

2.3. Study variables

Trained interviewers and medical staff assessed a wide range of covariates according to a standardized protocol (24). Anthropometry of waist circumference (WC) was measured at the midpoint between the inferior margin of the last rib and the iliac crest in the horizontal plane while the subject exhaled. Height and weight were measured to the nearest 0.1 cm and 0.1 kg, respectively, with participants wearing light clothing and being barefooted.

A questionnaire related to sociodemographic characteristics that included age, sex, smoking status, drinking status, regular exercise, living arrangement, and comorbidities was administered in the health interview. Smoking was defined as never (a person who has never smoked or has smoked less than five packs of cigarettes during their lifetime), former (a person who smoked more than five packs of cigarettes but who did not currently smoke), or current (a person who smokes more than five packs of cigarettes during their lifetime). Drinking was defined as the alcohol once or more times in a month. Regular exercise was defined as moderate exercise for 30 min, $\geq$ 5 times a week, vigorous exercise for 20 min, $\geq$ 3 times a week. Living arrangements were classified into two groups according to whether or not they

live alone. Comorbidities were defined as participants with at least one medical history of hypertension, dyslipidemia, stroke, myocardial infarction, angina, osteoarthritis, rheumatoid arthritis, kidney failure, diabetes mellitus, or cancer.

Dietary intake data were assessed using a one-day 24-h dietary recall method during the household interview. Trained dietitians interviewed the participants to recall and describe all the foods and beverages they had consumed in the previous day. Fish was classified according to the Composition Table of Marine Products in Korea 2018 of the National Institute of Fisheries Science (26), and amount of n-3 PUFA in individual fish, as g/day was calculated based on the Food Composition Table developed by the Korea Rural Development Administration in 2018 (27).

2.4. Statistical analyses

Descriptive analysis was conducted using clustering and stratifying variables, using a survey procedure that applied individual weights to the analysis (28). Continuous variables were analyzed using the independent sample t-test and are expressed as the mean ± standard error of the mean. Categorical variables were analyzed using the chi-square test and are expressed as frequencies and percentages.

Multiple regression models were used to determine unsuitable potential covariates and examine the association between the prevalence of LLM and dietary intake of n-3 PUFA and fish after adjusting for potential covariates. In multivariate models, covariates with $p < 0.20$ were selected as confounding factors and included in the fully adjusted model (29). The participants were subdivided into three groups according to tertiles of dietary n-3 PUFA and fish intake, separately (30). Analysis of covariance (ANCOVA) with Bonferroni correction was performed to assess the mean differences in ASM/BMI among the intake tertile groups after adjustment for confounding variables. The relationship between LLM and dietary intake of n-3 PUFA and fish was analyzed using multivariable logistic regression analysis. This analysis was used to obtain odds ratios (ORs) and 95% confidence intervals (CIs) adjusted for confounding variables. p-values for linear trends were estimated using the median values within each tertile of dietary intake, considering the unequal distances between tertiles. $p < 0.05$ was considered statistically significant. Statistical analysis was performed using SPSS 27.0 software (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Baseline characteristics of participants

Compared to those without LLM, men and women with LLM were older, had higher BMI, greater WC, increased prevalence of obesity and abdominal obesity, more comorbidities, consumed less alcohol, and had reduced energy intake (Table 1). There were no statistically significant differences in the prevalence of LLM, smoking status, living alone, and ALA intake between the LLM

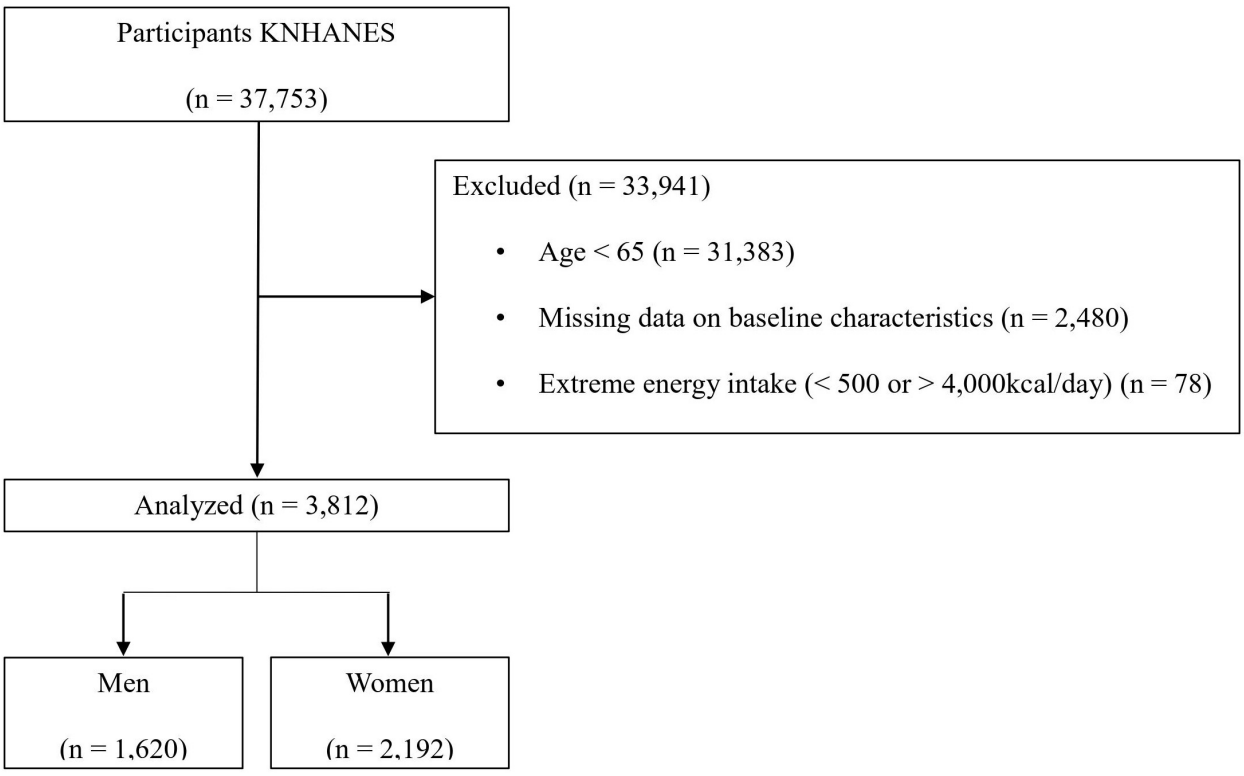

FIGURE 1
Flowchart of the inclusion and exclusion of participants.

TABLE 1 Baseline characteristics of men and women with and without low lean mass.

Variables	Men		*p*-Value*	Women		*p*-Value*
	Non-LLM (*n* = 1,167)	LLM (*n* = 453)		Non-LLM (*n* = 1,629)	LLM (*n* = 563)	
Age (year)	71.36 ± 0.13	73.04 ± 0.22	<0.001	71.81 ± 0.12	73.01 ± 0.19	<0.001
BMI (kg/m^2)	22.62 ± 0.08	24.29 ± 0.14	<0.001	23.46 ± 0.08	25.96 ± 0.14	<0.001
Obesity, *n* (%)	222 (19.0)	171 (37.7)	<0.001	495 (30.4)	335 (59.5)	<0.001
WC (cm)	83.46 ± 0.26	87.60 ± 0.42	<0.001	81.83 ± 0.23	87.10 ± 0.40	<0.001
Abdominal obesity, *n* (%)	285 (24.5)	185 (40.9)	<0.001	602 (37.1)	329 (58.9)	<0.001
Smoking status, *n* (%)			0.069			0.054
Never	200 (17.3)	74 (16.7)		1,446 (90.1)	501 (90.6)	
Former	646 (56.0)	275 (61.9)		69 (4.3)	31 (5.6)	
Current	308 (26.7)	95 (21.4)		90 (5.6)	21 (3.8)	
Drinking status, *n* (%)	684 (59.3)	224 (50.5)	0.005	279 (17.4)	72 (13.0)	0.037
Regular exercise, *n* (%)	260 (22.3)	88 (19.4)	0.305	322 (19.8)	68 (12.1)	<0.001
Living alone, *n* (%)	80 (6.9)	30 (6.7)	0.957	418 (25.7)	142 (25.3)	0.214
Comorbidities	720 (62.1)	350 (78.5)	< 0.001	1,289 (80.1)	493 (88.4)	<0.001
Dietary intake						
Energy intake (kcal/day)	1,897.07 ± 17.56	1,710.18 ± 26.21	< 0.001	1,459.16 ± 12.41	1,365.54 ± 19.94	<0.001
EPA+DHA (g/day)	0.84 ± 0.04	0.76 ± 0.06	0.830	0.50 ± 0.02	0.40 ± 0.03	0.002
ALA (g/day)	1.47 ± 0.06	1.24 ± 0.08	0.393	1.14 ± 0.07	1.01 ± 0.06	0.088
Fish (g/day)	43.56 ± 2.21	36.83 ± 3.03	0.545	26.44 ± 1.37	18.65 ± 1.74	<0.001

Values are expressed as mean ± standard error of the mean for continuous variables or as number (percentage) for categorical variables. *p-values were calculated using independent sample t-test for continuous variables; chi-square test for categorical variables LLM, low lean mass; BMI, body mass index; WC, waist circumference; PUFA, polyunsaturated fatty acid; EPA, Eicosapentaenoic acid; DHA, Docosahexaenoic acid; ALA, alpha linolenic acid.

and non-LLM groups. Women with LLM exercised less regularly and consumed less EPA, DHA, and fish than women without LLM. The total population with LLM was older, had higher BMI, greater WC, increased prevalence of obesity and abdominal obesity, more comorbidities, reduced exercise activities, and consumed less alcohol, energy, EPA, DHA, and fish than those without LLM (**Supplementary Table 1**).

3.2. Associations between prevalence of LLM and intakes of n-3 PUFA and fish

Logistic regression analysis revealed that the prevalence of LLM was negatively associated with the intake of EPA and DHA, and fish, but not ALA in women, after adjusting for potential confounders (**Table 2**). However, there was no significant association between the prevalence of LLM and the intake of n-3 PUFA and fish in men. After full adjustment, the intake of EPA and DHA and fish were also negatively associated in the total study population (**Supplementary Table 2**). We divided women into 4 groups; non-sarcopenic non-obesity, sarcopenic non-obesity, non-sarcopenic obesity, and sarcopenic obesity. The prevalence of LLM was significantly associated with intake of EPA and DHA, and fish in women with non-sarcopenic non-obesity, sarcopenic non-obesity, and sarcopenic obesity (**Supplementary Table 3**).

3.3. Associations between muscle mass and intakes of dietary n-3 PUFA and fish

After adjusting for potential confounders, ANCOVA revealed a significant positive association between muscle mass and intake of EPA, DHA, and fish, but not ALA in women, as a continuous and non-continuous variable (**Table 3**). However, there were no associations between muscle mass and intakes of n-3 PUFA and fish in men (**Table 3**) and the total population (**Supplementary Table 4**).

4. Discussion

The present study demonstrates that consumption of EPA, DHA, and fish were negatively associated with the prevalence of LLM and positively correlated with muscle mass in Korean older women, but not in men, after adjusting for potential confounders in KNHANES data from 2008 to 2011. Consistent with the present study, a higher total n-3 PUFA intake was associated with a lower risk of sarcopenia among kidney transplant patients in Brazil (5). The risk of sarcopenia was also negatively associated with serum levels of total n-3 PUFA and decreased by 71% with each standard deviation increment of serum level of total n-3 PUFA in Korean older adults (6). Yang et al. (7) also showed that the ratio

TABLE 2 Associations between prevalence of low lean mass and n-3 PUFA and fish intake in men and women.

Variables	Tertiles of n-3 PUFA and fish intake			*p*-Trend
	T1	T2	T3	
Men				
EPA+DHA (g/day), range	<0.13	0.13 ≤ to < 0.67	≥0.67	0.522
No. with/without LLM	155/385	168/372	130/410	
OR (95% CI)	1	1.214 (0.881 – 1.673)	0.962 (0.673– 1.376)	
ALA (g/day), range	< 0.58	0.58 ≤ to < 1.30	≥ 1.30	0.404
No. with/without LLM	175/365	156/384	122/418	
OR (95% CI)	1	0.897 (0.657 – 1.225)	0.842 (0.582 – 1.217)	
Fish (g/day), range	< 0.24	0.24 ≤ to < 34.20	≥ 34.20	0.567
No. with/without LLM	152/388	169/371	132/408	
OR (95% CI)	1	1.375 (0.983 – 1.922)	1.038 (0.748 – 1.442)	
Women				
EPA+DHA (g/day), range	< 0.06	0.06 ≤ to < 0.40	≥ 0.40	0.002
No. with/without LLM	207/523	195/536	161/570	
OR (95% CI)	1	1.088 (0.818 – 1.447)	0.654 (0.478–0.896)	
ALA (g/day), range	< 0.43	0.43 ≤ to < 0.95	≥ 0.95	0.602
No. with/without LLM	189/541	194/537	180/551	
OR (95% CI)	1	1.272 (0.940 – 1.722)	1.151 (0.829–1.597)	
Fish (g/day), range	< 0.00	0.00 ≤ to < 15.33	≥ 15.33	<0.001
No. with/without LLM	239/626	165/431	159/572	
OR (95% CI)	1	1.214 (0.926 – 1.592)	0.590 (0.423–0.823)	

Odds ratios (ORs) and 95% confidence intervals (CIs) were presented. The logistic regression model was adjusted for age, abdominal obesity, alcohol consumption, comorbidities, and energy intake for men and for age, abdominal obesity, smoking, regular exercise, and energy intake for women. LLM, low lean mass; PUFA, polyunsaturated fatty acid; EPA, Eicosapentaenoic acid; DHA, Docosahexaenoic acid; ALA, alpha linolenic acid.

of daily total n-3 PUFA intake to energy intake was negatively associated with the risk of sarcopenic obesity in older Korean women, but not in men.

Dos Reis et al. (5) observed that the intake of total n-3 PUFA was positively associated with muscle mass, estimated by ASM divided by the height squared, in kidney transplant patients. The Maastricht Sarcopenia Study of a community-dwelling population found that older adults with sarcopenia had a significantly lower intake of total n-3 PUFA and lower erythrocyte EPA levels than those without sarcopenia (12). A meta-analysis of clinical trials reported that supplementation with EPA and DHA elicited an approximately 0.33 kg increase in muscle mass in older adults, especially when more than 2 g/day of EPA and DHA was administered (17). n-3 PUFA increases the rate of muscle protein synthesis by stimulating the mTOR signaling pathway, and prevents muscle degradation by reducing inflammatory signaling pathway signaling (18).

Epidemiologic studies have consistently reported that the intake of total n-3 PUFA, EPA, and DHA was associated with muscle function measured by leg strength, time to rise from a chair, timed up-and-go, gait speed, handgrip strength, and SPPB in American older adults (8), Japanese men (9), Finnish women (10), and Korean women (11). Similarly, high plasma concentrations of total n-3 PUFA, EPA, and DHA were associated with gait speed, handgrip strength, and SPPB among older adults in a Three-City study (13) as well as in Korea (6), United States (15), and Italy (14). In particular, our previous study showed that erythrocyte EPA and DHA levels are associated with gait speed in Korean older adults (16). A meta-analysis of clinical trials found that supplementation with EPA and DHA improved gait speed in older adults (17). However, Rossato et al. (31) reported that intake of EPA and DHA was not associated with muscle strength or voluntary peak isokinetic knee extensor strength in American older adults, and their average intake of EPA and DHA was 0.1 g/day. On the other hand, the average intake of EPA and DHA was 0.6 g/day among Korean older adults in the present study, which was six times higher than that in Americans. The Food and Agriculture Organization of the United Nations reported that the intake of aquatic food, a rich source of n-3 PUFA, was more than 50 kg per capita per year among Koreans, the highest in the world, and only 20-30 kg per capita per year among Americans (32).

To our knowledge, no study has investigated the association between fish intake and muscle mass. However, previous studies have reported that fish intake is positively associated with muscle function. Muscle function measured by grip strength was positively associated with consumption of fatty fish and oily fish, but not white fish and shells, in older adults from the Hertfordshire cohort (19) and from the UK Biobank study (20). In addition, a Mediterranean diet, known to contain many fish, was negatively associated with the risk of sarcopenia and positively associated with gait speed in older Iranian adults (22). The Finnish Osteoporosis Risk Factor and Prevention Fracture Prevention Study also found that the Mediterranean diet score was significantly associated with gait speed in older women (21). Rondanelli et al. (33) suggested

TABLE 3 Correlation between muscle mass and n-3 PUFA and fish intake in men and women.

Variables (g/day)		Tertiles of n-3 PUFA and fish intake			*p*-Trend*	Continuous	
		T1	T2	T3		*r*	*p*-Value
Men	EPA+DHA, range	< 0.13	0.13 ≤ to < 0.67	≥ 0.67			
	ASM/BMI	0.85 ± 0.004	0.84 ± 0.004	0.85 ± 0.004	0.743	0.012	0.689
	ALA, range	< 0.58	0.58 ≤ to < 1.30	≥ 1.30			
	ASM/BMI	0.84 ± 0.004	0.84 ± 0.004	0.86 ± 0.004	0.754	0.017	0.603
	Fish, range	< 0.24	0.24 ≤ to < 34.20	≥ 34.20			
	ASM/BMI	0.85 ± 0.004	0.83 ± 0.004	0.85 ± 0.004	0.520	0.027	0.316
Women	EPA+DHA, range	< 0.06	0.06 ≤ to < 0.40	≥ 0.40			
	ASM/BMI	0.56 ± 0.003	0.56 ± 0.003	0.57 ± 0.003	0.026	0.054	0.013
	ALA, range	< 0.43	0.43 ≤ to < 0.95	≥ 0.95			
	ASM/BMI	0.56 ± 0.003	0.56 ± 0.003	0.57 ± 0.003	0.445	<0.001	0.964
	Fish, range	< 0.00	0.00 ≤ to < 15.33	≥ 15.33			
	ASM/BMI	0.55 ± 0.003	0.56 ± 0.003	0.57 ± 0.003	0.005	0.083	<0.001

*p-Trend for the differences in muscle mass (ASM/BMI) according to tertiles of n-3 PUFA and seafood intake after adjustment for confounding factors, including age, abdominal obesity, drinking, comorbidities, and energy intake for men and age, abdominal obesity, smoking, regular exercise, and energy intake for women using ANCOVA test with Bonferroni correction. Values represent correlations (r). PUFA, polyunsaturated fatty acid; EPA, Eicosapentaenoic acid; DHA, Docosahexaenoic acid; ALA, alpha linolenic acid.

that fish contain anti-sarcopenic compounds, such as n-3 PUFA, proteins, vitamin D, magnesium, and carnitine, which could reduce inflammation and improve muscle response to exercise and diet.

The Osteoporosis Risk Factor and Prevention Fracture Prevention Study reported that ALA intake was positively associated with muscle function, but not muscle mass, among Finnish older women (10). The Maastricht Sarcopenia Study analysis found that ALA intake was significantly associated with muscle function, but erythrocyte levels of ALA, a marker for dietary intake, were not associated with muscle function in Dutch older adults (12). In addition, supplementation of 14 g/day of ALA with a resistance training program increased knee flexor muscle thickness and had an effect on muscle functions, such as chest and leg press, in Canadian older men (23). However, the increased muscle thickness might not be due to the intake of ALA but to exercise, since all participants in the trial were on resistance training programs (23). Thus, the documented effect of ALA intake on muscle function is inconsistent and might not be associated with muscle mass, which supports the results of the present study.

A noteworthy point of our findings was that intake of EPA, DHA, and fish was associated with the prevalence of LLM and muscle mass in older women, but not in older men. Consistent with the present study, a higher ratio of daily total n-3 PUFA intake to energy intake was negatively associated with the risk of sarcopenic obesity (7), and intake of EPA and DHA was positively associated with handgrip strength (11) in Korean older women, but not in men, suggesting that intake of n-3 PUFA might be beneficial for sarcopenia among Korean women. In the present study, intakes of EPA and DHA, and ALA were analyzed separately instead of n-3 PUFA, and appendicular skeletal muscle mass was evaluated instead of handgrip strength. Asian people, especially Asian women, tend to have lower muscle mass and higher body fat mass with central adiposity than Western populations (34, 35). In the present study, the average BMI and WC of sarcopenic women were 26 kg/m^2 and 87 cm, respectively, indicating that sarcopenic women were mostly abdominal obese, but sarcopenic men were not. Thus, in the present study, muscle mass was calculated based on ASM divided by BMI, but not by height squared (m^2), to consider sarcopenic obesity. Previous epidemiological studies have shown that patients with sarcopenic obesity have higher levels of cytokines, such as C-reactive protein (CRP), interleukin-6 (IL-6), and monocyte chemotactic protein-1 than those with sarcopenia (36–38). Inflammation is a well-known component of the pathophysiology of muscle wasting (39). A meta-analysis of clinical trials showed that n-3 PUFA supplementation reduced inflammatory biomarkers such as CRP and IL-6 in older adults (40). Al-Safi et al. (41) further reported that supplementation with EPA and DHA decreased the levels of IL-1 and tumor necrosis factor-alpha (TNF-α), and the reduction in cytokines was greater among obese women than among normal-weight women, suggesting that n-3 PUFA could be more beneficial for those with inflammation. Additionally, women in general have a greater capacity to convert ALA to DHA than men due to estrogen; thus, the plasma level of DHA is higher in women than in men (42). Canon et al. (43) reported that estrogen decreases the levels of CRP and IL-6 and inhibits chronic inflammation. Furthermore, Smith et al. (44) observed that supplementation of EPA and DHA enhanced muscle anabolism when plasma leucine concentrations were clamped at 165-175 μmol/L in healthy adults. However, McGlory et al. (45) found that supplementation with EPA and DHA had no effect on muscle protein synthesis during peak plasma leucine concentrations of 250-300 μmol/L achieved by the ingestion of 30 g of whey protein in young men. It is possible that ingestion of 30 g of whey protein could maximize the rate of muscle protein synthesis to the extent that fish oil supplementation would not have exerted a further anabolic influence (46, 47). Thus, previous studies have suggested that a beneficial effect of n-3 PUFA on muscle synthesis might be observed when protein is insufficient (44–47). In the present study, the average protein intake was 62 g/day in men aged 65 years or older, similar to the Korean dietary reference intake (KDRIs) of 60 g/day, but the average protein intake was 45 g/day in women aged 65 years or older, which is lower than the KDRIs

of 50 g/day (48, 49). Additionally, protein intake was lower among older men with LLM (57 g/day vs. 63 g/day; $p = 0.002$) and women with LLM (43 g/day vs. 45 g/day; $p = 0.015$) than those without LLM, although protein intake as g/kcal/day was not different between men with and without LLM (33 mg/kcal vs. 33 mg/kcal; $p = 0.573$) and between women with and without LLM (31 mg/kcal vs. 31 mg/kcal; $p = 0.460$). As Korean women consume insufficient amounts of protein, the beneficial effect of n-3 PUFA on LLM might be observed only in Korean women.

The major strength of the present study was that the data were gathered from a nationally representative survey throughout Korea; thus, the findings can be generalized to older adults in Korea. However, some limitations should be considered when interpreting the results of this study. First, the cross-sectional study design was unable to establish a causal relationship between the prevalence of LLM and the intake of n-3 PUFA and fish. Second, muscle strength or performance were not measured in the KNHANES 2008-2011, and sarcopenia could not be diagnosed. However, loss of muscle mass with aging is clinically important because it leads to diminished strength and exercise capacity (50). Third, the dietary intake of one day was assessed using the 24-h recall method, which could have recall bias and did not reflect the usual dietary intake.

5. Conclusion

The present study demonstrates that consumption of high levels of EPA, DHA, and fish could have beneficial effects on the prevention of LLM by improving muscle mass in older women. Further studies are needed to verify the preventive effects of EPA, DHA, and fish consumption on sarcopenia in large population-based longitudinal studies of diverse ethnic origins.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: https://knhanes.kdca.go.kr/knhanes/sub03/sub03_01.do.

Ethics statement

The studies involving human participants were reviewed and approved by Institutional Review Board of Hanyang University (HYUIRB-202208-003). The patients/participants provided their written informed consent to participate in this study.

Author contributions

YK performed statistical analyses and wrote the manuscript. YP designed the study, revised the manuscript, and was responsible for this work. Both authors have read and agreed to the published version of the manuscript.

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Conflict of interest

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.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2023.1119719/full#supplementary-material

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Association between sarcopenia and kidney stones in United States adult population between 2011 and 2018

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Purpose: To investigate the relationship between kidney stones and sarcopenia in United States adult population between 2011 and 2018.

Materials and methods: We conducted a cross-section study based on the National Health and Nutrition Examination Survey (NHANES) including 39,156 individuals. Sarcopenia was assessed by the sarcopenia index. Association between kidney stones and sarcopenia verified by multiple logistic regression analysis and dose–response curves analysis using restricted cubic spline (RCS) regression. Meanwhile, propensity score matching (PSM) was performed to exclude the effect of confounding variables.

Results: There were 9,472 participants in the study by our accurate enrollment screening process. The odds of kidney stones decreased significantly with the increase of sarcopenia index. Logistic regression analysis showed that sarcopenia expressed significant differences in the participants which suffered kidney stone before PSM ($p<0.001$). In model 4, adjusting all relevant covariates shown that adjusted odds ratio (aOR) of the 95% confidence intervals for kidney stones in all participants, age <39years and age ≥40 years, were, respectively, 1.286 (1.006–1,643), 1.697 (1.065–2.702), and 0.965 (0.700–1.330) for sarcopenia, and p values were 0.044, 0.026, and 0.827. After performing PSM, the aOR of the 95% in modal 4 for kidney stones in all participants and age <40year were 2.365 (1.598–3.500) and 6.793 (2.619–17.6180), respectively ($p<0.01$), and especially the aOR in participants (age ≥40) was 1.771(1.138–2.757) with p value being 0.011.

Conclusion: Sarcopenia was positively related to the potential risk of kidney stones in the United States adult population.

KEYWORDS

sarcopenia, sarcopenia index, kidney stones, cross-section study, NHANES

Introduction

Urolithiasis was the most known urology disease around the worlds, and it caused a certain problem of various groups of patients. Kidney stones as the most common type of urolithiasis had an increasing prevalence over the past decades, placing high costs and clinical burdens on the healthcare system (1). In the United States, kidney stones spent more than $2.1 billion on healthcare in 2000 (2). Kidney stones formation had been shown to be related to environmental and genetic factors such as climate, diet, fluid intake, smoking, caffeine, age, gender, body mass index (BMI), and type 2 diabetes (DM) (3).

Sarcopenia had been defined as a progressive and systemic skeletal muscle disease associated with accelerated loss of muscle mass and function. More recently, sarcopenia had been defined as a disease with many adverse effects such as falls, functional decline, weakness, and death (4). In 2018, EWGSOP2 updated the definition and diagnostic guidelines for sarcopenia, stating that people with low muscle strength, muscle mass/mass, and physical performance would be diagnosed with sarcopenia (5). Interestingly, we found a strong correlation between sarcopenia and stones, and it had not been reported in any study.

The purpose of this study was to investigate the exposure-response relationship between sarcopenia and the incidence of kidney stones in the National Health and Nutrition Examination Survey (NHANES) from 2011 to 2018.

Materials and methods

Study design and participants

NHANES was a program that assessed the health and nutritional status of the American people, which combined interviews and physical examinations to collect data focusing on diet and health-related incidents. The study protocol was endorsed by the NHANES Institutional Review Board, and all participants provided informed consent during the survey. In this study, there were 39,156 participants in eight NHANES cycles 2011–2012, 2012–2013, 2013–2014, 2014–2015, 2015–2016, 2016–2017, and 2017–2018. The study excluded participants if their conditions met any of the following: (1) Their age was under 20 years old (n = 22,617); (2) They had no kidney stones or sarcopenia information (n = 10,815); (3) Their marital status, BMI, education, hypertension, and diabetes were unknown (n = 10,285); (4) Their blood urea nitrogen, creatinine and uric acid were unknown (n = 9,472). Finally, we collected 9,472 participants, as shown in Figure 1.

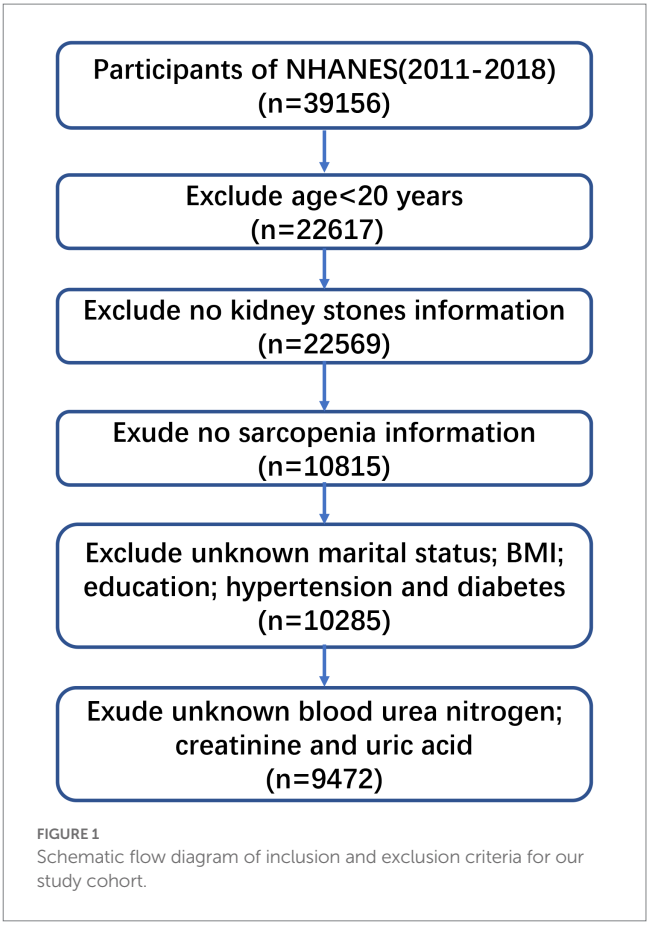

FIGURE 1
Schematic flow diagram of inclusion and exclusion criteria for our study cohort.

Exposure variable and outcomes variable

Sarcopenia was the major exposure variable in this study. Sarcopenia was assessed by the sum of the muscle mass of the four limbs (ALM, appendicular lean mass). Dual-energy X-ray absorptiometry (DEXA) was used to measure ALM by NHANES. Pregnant participants and those participants who weight more than 136.4 kg or height more than 192.5 cm were excluded from the study, because these individuals could not be measured by DEXA. We calculated the sarcopenia index as following: sarcopenia index = total appendicular skeletal muscle mass (in kg)/BMI (kg/m^2). Sarcopenia was defined by sarcopenia index: it judged to exist sarcopenia if sarcopenia index of men and women was less than 0.789 and 0.512, respectively.

The major outcome variable of the study was the kidney stones history. The kidney stones history was assessed by the answers of participants. The participants who had suffered kidney stones were divided into kidney stones groups, and the rest of participants were divided into non-kidney stones groups.

Potential covariates

Based on previous studies, relevant covariates were identified. Continuous variables included age (<40 years/≥40 years); body mass index (BMI); blood urea nitrogen; and creatinine and uric acid. Categorical variables included Gender (male/female); Race (non-Hispanic white/non-Hispanic black/Mexican American/other Hispanic/other); Education level (less than high school/high school or equivalent/college or above); Marital status (married/unmarried); Hypertension; Smoking status (never/former/current); Alcohol use; Vigorous recreational activities; Moderate recreational activities; Sarcopenia; blood urea nitrogen; creatinine; and uric acid.

Statistical analysis

Continuous variables and categorical variables were presented as mean ± standard deviation and number (percentage), respectively.

Comparisons among different groups were performed by *t*-tests and one-way ANOVA tests for normally distributed continuous variables, then non-normal continuous variables was compared by independent-samples Kruskal–Wallis tests, and categorical variables among different groups were determined statistical differences by Chi-square tests. Logistic regression analyzed the relationship between sarcopenia and the presence of kidney stones, using the corrected odds ratio (OR) and corresponding 95% confidence intervals (CI) to describe the associations. In the extended model, model 1 was univariate analysis; model 2 was modified gender, age, and race; model 3 was model 2 plus education level, marital status, and BMI; model 4 was model 3 adding hypertension, smoking status, alcohol use, physical activities, blood urea nitrogen, creatinine, and uric acid.

The powerful tool of restricted cubic spline (RCS) function was applied to describing dose–response relationships between continuous variables and outcomes, and was also utilized in our research to characterize the dose–response relationship among sarcopenia index and kidney stone risk, adjusted for model variables. All statistical analyses were conducted using IBM SPSS 20.0 software (IBM, United States) and GraphPad Prism8 software (GraphPad Software Inc., La Jolla, CA, United States). *p*-Values less than 0.05 were considered to be statistically significant.

Results

Participants' characteristics

As shown in Figure 1, 39,156 participants were recruited from the NHANES (2011–2018). During screening, basic characteristics of participants are summarized in Table 1. Eight thousand seven hundred and thirteen (92%) participants were divided into none-stone formers group and 759 (8%) into stone formers. Gender and education level had no significance between the two groups ($p > 0.05$). The others had significant differences between both groups, including age, race, marital status, BMI, hypertension, smoking status, alcohol use, vigorous recreational activities, moderate recreational activities, sarcopenia, blood urea nitrogen, creatinine, and uric acid ($p < 0.05$). Supplementary Table S1 summarizes basic characteristics of participant with no-sarcopenia group and sarcopenia group. Eight thousand six hundred and sixty-one (91.4%) participants were divided into no-sarcopenia and 811 (8.6%) into sarcopenia. We found that people with sarcopenia were more likely to be elderly (age ≥ 40 years), married, Mexican American, college degree or above, higher BMI, less vigorous recreational activities, less moderate recreational activities, lower creatinine, and higher uric acid ($p < 0.05$).

Sarcopenia and kidney stones

Figure 2 shows the dose–response relationships between sarcopenia index and kidney stones. There was a no-linear association between LMA (lumbar muscle area) and the prevalence of kidney stones. It showed that the prevalence of kidney stones decreased with increase of sarcopenia index. Then, logistic regression analysis further confirmed that sarcopenia was positively related with prevalence of kidney stones (Table 2). The adjusted odds ratio (aOR) of all participants was 1.620 (95% CI, 1.290–2.033), 1.458 (95% CI, 1.152–1.846), 1.287 (95% CI, 1.010–1.639), and 1.286 (95% CI, 1.006–1.643), respectively ($p < 0.05$). The adjusted odds ratio (aOR) of participants (<40 years) was 1.955 (1.304–2.930), 2.089 (1.377–3.169), 1.926 (1.254–2.959), and 1.697 (1.065–2.702), respectively ($p < 0.05$). However, there were no significant differences about aOR on participants (≥40 years). There were adjusted covariate in four models: model 1: univariate analysis; model 2: gender, age and race; model 3: model 2 plus education level, marital status, and BMI; and model 4: model 3 plus hypertension, smoking status, alcohol use, physical activities, blood urea nitrogen, creatinine, and uric acid.

Association after propensity score matching

Participants in the study were performed propensity score matching, because of differences between none-stone formers group and formers group. The results after propensity score matching are shown in Figures 3, 4. After propensity score matching, logistic regression analysis clearly shown that sarcopenia was positively related with prevalence of kidney stones. Table 3 displays that *p*-values were less than 0.05 among participants (all, <40 years and ≥40 years) in four models. The adjusted odds ratio (aOR) of all participants was 2.325 (1.602–3.376), 2.300 (1.572–3.366), 2.342 (1.588–3.455), and 2.365 (1.598–3.500), respectively ($p < 0.01$). Then, the adjusted odds ratio (aOR) of participants (<40 years) was 5.600 (2.287–13.710), 5.945 (2.362–14.964), 6.334 (2.479–16.180), and 6.793 (2.619–17.618), respectively ($p < 0.01$). Finally, the adjusted odds ratio (aOR) of participants (≥40 years) was 1.787 (1.173–2.723), 1.761 (1.148–2.700), 1.756 (1.132–2.723), and 1.771 (1.138–2.757), respectively ($p < 0.05$). There were adjusted covariates in four models: model 1: univariate analysis; model 2: gender, age, and race; model 3: model 1 plus education level, marital status, and BMI; and model 4: model 3 plus hypertension, smoking status, alcohol use, physical activities, blood urea nitrogen, creatinine, and uric acid.

Figure 4 shows the dose–response relationships between sarcopenia index and kidney stones after propensity score matching. There was a no-linear association between sarcopenia index and the prevalence of kidney stones. Respectively comparing Figures 4, 2, Table 2, 3, it was proved that sarcopenia had more dramatic impact on the prevalence of kidney stones especially after PSM. With increasing of sarcopenia index, participants had lower prevalence of kidney stones.

Discussion

Kidney stones caused a series of damages to patient health and brought great social, economic, and healthy burden, due to its high incidence and recurrence rate. Therefore, it was essential for us to discover the risk factors of kidney stones, and the risk factors might help us to decrease the recurrence of stone disease and reduce burden of society and medicine. Our study discussed the relationship between sarcopenia and kidney stones. Firstly, we explored the association between sarcopenia and kidney stones. Then, we concluded that the odds of kidney stones decreased significantly with the increase of sarcopenia index which was negatively related to sarcopenia, and it was proved by the dose–response curve. However, we found that

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TABLE 1 Baseline characteristics of participants between 2011 and 2018.

Characteristic	Total	None-stone formers	Stone formers	*p*-Value
	N (%)	*N* (%)	*N* (%)	
Total patients	9,472	8,713 (92.0)	759 (8.0)	
Gender				0.587
Male	4,657 (49.2)	4,291 (49.2)	366 (48.2)	
Female	4,815 (50.8)	4,422 (50.8)	393 (51.8)	
Age				<0.001
<40 years	4,793 (50.6)	4,518 (51.9)	275 (36.2)	
≥40 years	4,679 (49.4)	4,195 (48.1)	484 (63.8)	
Race				<0.001
Non-Hispanic white	3,428 (36.2)	3,055 (35.1)	373 (49.1)	
Non-Hispanic black	1934 (20.4)	1837 (21.1)	97 (12.8)	
Mexican American	1,374 (14.5)	1,274 (14.6)	100 (13.2)	
Other Hispanic	948 (10.0)	856 (9.8)	92 (12.1)	
Other	1788 (18.9)	1,691 (19.4)	97 (12.8)	
Education level				0.695
Less than high school	1,610 (17.0)	1,479 (17.0)	131 (17.3)	
High school or equivalent	2063 (21.8)	1907 (21.9)	156 (20.6)	
College or above	5,799 (61.2)	5,327 (61.1)	472 (62.2)	
Marital status				0.011
Married	4,625 (48.8)	4,221 (48.4)	404 (53.2)	
Unmarried	4,847 (51.2)	4,492 (51.6)	355 (46.8)	
BMI (kg/m^2)				<0.001
<25.0	3,001 (31.7)	2,835 (32.6)	166 (21.9)	
25.0–29.9	2,966 (31.3)	2,731 (31.4)	235 (31.0)	
≥30.0	3,497 (37.0)	3,139 (36.1)	358 (47.2)	
Hypertension				<0.001
Yes	2,223 (23.5)	1945 (22.3)	278 (36.6)	
No	7,249 (76.5)	6,768 (77.7)	481 (63.4)	
Smoking status				<0.001
Never	5,733 (60.5)	5,332 (61.2)	401 (52.8)	
Former	1,613 (17.0)	1,457 (16.7)	156 (20.6)	
Current	2,126 (22.4)	1924 (22.1)	202 (26.6)	
Alcohol use				0.010
Yes	7,040 (74.3)	6,446 (74.0)	594 (78.3)	
No/Unknown	2,432 (25.7)	2,267 (26.0)	165 (21.7)	
Vigorous recreational activities				<0.001
Yes	2,967 (31.3)	2,779 (31.9)	188 (24.8)	
No	6,505 (68/7)	5,934 (68.1)	571 (75.2)	
Moderate recreational activities				0.042
Yes	4,277 (45.2)	3,961 (45.5)	316 (41.6)	
No	5,195 (54.8)	4,752 (54.5)	443 (58.4)	
Sarcopenia				<0.001
Yes	811 (8.6)	715 (8.2)	96 (12.6)	
No	8,661 (91.4)	7,998 (91.8)	663 (87.4)	
Blood urea nitrogen (mg/dl)	12.53 ± 4.50	12.48 ± 4.40	13.16 ± 5.42	<0.001
Creatinine (mg/dl)	0.85 ± 0.37	0.85 ± 0.34	0.89 ± 0.58	<0.001
Uric acid (mg/dl)	5.32 ± 1.39	5.32 ± 1.39	5.35 ± 1.39	<0.001

For categorical variables, *p* values were analyzed by Chi-square tests. For continuous variables, the *t*-test was used. BMI, body mass index.

sarcopenia was not independent risk factor of kidney stones in participants (≥40 years). Therefore, PSM was performed to exclude the effects of other variables. After performing PSM, all participants showed that sarcopenia had the positively impact on prevalence of kidney stones, and the aOR in participants (age ≥40) in model 4 was 1.771 (1.138–2.757). In conclusion, sarcopenia was the independent hazard factor of kidney stones.

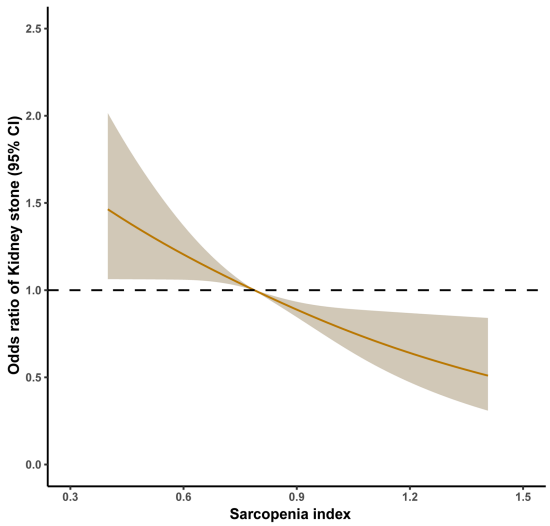

FIGURE 2
Relative risk for kidney stones based on sarcopenia index before PSM. The shaded areas represent upper and lower 95% CIs. Adjustment factors are as same as which presented in extended model 4. Restricted cubic spline (RCS) plot of the association between sarcopenia index and kidney stones. The solid and dashed lines represent the odds ratios and 95% confidence intervals.

Risk factors of kidney stones had increased around the world, and it might influence the more diagnosis of kidney stone. The formation of kidney stones was closely related to risk factors such as age, gender, dietary structure, environmental factors, genetics, abnormal urinary anatomy, and infection (3). Especially, higher rates of obesity; diabetes; more intake of salt and animal protein; higher consumption of sugary beverages; global warming; less exercise; and sedentary behavior might contribute to higher incidence and prevalence of kidney stones (6–8). In recent years, studies had found a positive correlation between BMI, waist circumference, and the risk of kidney stones (9). In general, as BMI increases, so does visceral obesity and hepatic steatosis, both of which were associated with low levels of urinary PH, and low urinary PH could lead to the development of uric acid stones (10). Adipose tissue, as an endocrine organ, was a source of adipokines and inflammatory cytokines that can lead to insulin resistance, inflammatory, and enhanced oxidative stress states, and these conditions would lead to stone crystal formation (11, 12). Sarcopenia was defined as a progressive and systemic skeletal muscle disease involving an accelerated loss of muscle mass and function, which was associated with increased adverse outcomes, including falls, functional decline, weakness, and death (13). Age-related mechanisms that promoted sarcopenic episodes including inflammation, immune aging, anabolic resistance, and increased oxidative stress (14). We found that the pathophysiological mechanisms of sarcopenia and kidney stones shared some of the same risk factors, such as obesity, insulin resistance, lack of physical activity, and chronic inflammation etc. In obese patients, ectopic deposition of fat in the liver and skeletal muscle caused an inflammatory response and insulin resistance, and fat tissue secreted adipokines and cytokines induced a decrease in skeletal muscle mass and function, leading to the development of sarcopenia (14–16). When insulin resistance occurred, the gluconeogenesis process in myocytes was promoted, resulting in

TABLE 2 Logistic regression analyzed the relationship between sarcopenia and the presence of kidney stones.

	Model 1		Model 2		Model 3		Model 4	
	aOR (95% CI)	*p*	aOR (95% CI)	*p*	aOR (95% CI)	*p*	aOR (95% CI)	*p*
All participants								
Sarcopenia								
Yes	1.620 (1.290–2.033)		1.458 (1.152–1.846)		1.287 (1.010–1.639)		1.286 (1.006–1.643)	
No	Reference		Reference		Reference		Reference	
p for trend	<0.001		0.002		0.041		0.044	
Age <40 years								
Sarcopenia								
Yes	1.955 (1.304–2.930)		2.089 (1.377–3.169)		1.926 (1.254–2.959)		1.697 (1.065–2.702)	
No	Reference		Reference		Reference		Reference	
p for trend	0.001		0.001		0.003		0.026	
Age ≥40 years								
Sarcopenia								
Yes	1.311 (0.995–1.727)		1.270 (0.955–1.687)		1.085 (0.810–1.454)		0.965 (0.700–1.330)	
No	Reference		Reference		Reference		Reference	
p for trend	0.054		0.100		0.584		0.827	

Adjusted covariates: model 1: univariate analysis; model 2: gender, age and race; model 3: model 2 plus education level, marital status, and BMI; model 4: model 3 plus hypertension, smoking status, alcohol use, physical activities, blood urea nitrogen, creatinine and uric acid. BMI, body mass index; CI, confidence interval; aOR, adjusted odds ratio.

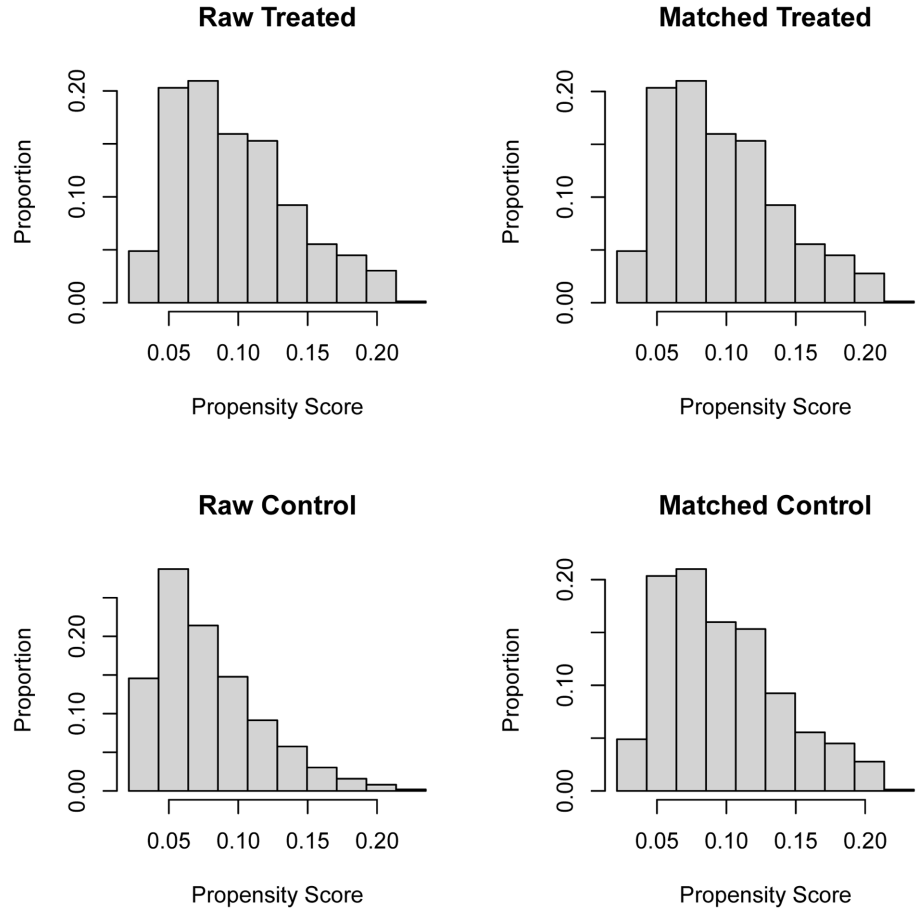

FIGURE 3
Distribution of propensity score before and after matching.

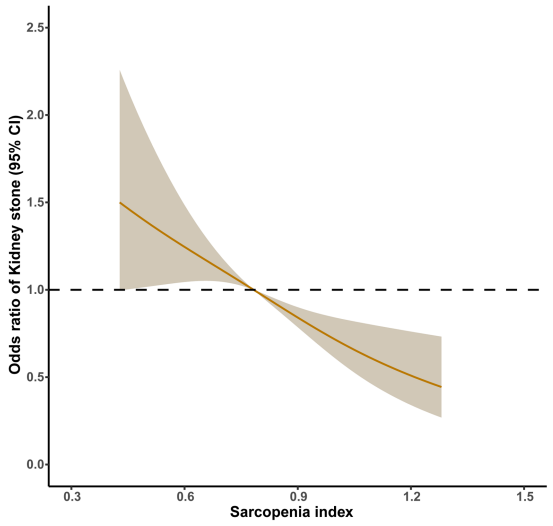

FIGURE 4
Relative risk for kidney stones based on sarcopenia index after PSM. The shaded areas represent upper and lower 95% CIs. Adjustment factors are as same as which presented in extended model 4. Restricted cubic spline (RCS) plot of the association between sarcopenia index and kidney stones. The solid and dashed lines represent the odds ratios and 95% confidence intervals.

decreased protein synthesis and increased catabolism, which lead to decreased muscle mass (17). Then, for lack of physical activity, it lead to abdominal fat deposition, systemic inflammatory response, and insulin resistance, further reducing the body's lipid and glucose oxidation (18). Finally, some studies had found that patients with sarcopenia also have higher levels of inflammatory cytokines and inflammatory indicators that were involved in the activation of apoptosis, leading to a decrease in myofilament protein synthesis, which lead to sarcopenia (19). It shown in our study that preliminary confirmation was published about the relationship between sarcopenia and the prevalence of kidney stones. Sarcopenia might contribute to the development and progression of kidney stones through increasing obesity, insulin resistance, inflammation, and decreasing recreational activities.

In conclusion, we proved the associations between sarcopenia and the odds of kidney stones while controlling for potential variables, though several limitations still existed. First of all, we could not be completely convinced of the relationship between sarcopenia and kidney stones, because the study was a cross-sectional study. It needed more exploration to verify. Then, there was no consideration about relationship between position, type, and size of kidney stone and sarcopenia, and the basic characteristics of kidney stones was useful for prevention of kidney stones. Therefore, we should perform more studies to explore the potential mechanisms and to identify causality.

TABLE 3 Logistic regression analyzed the relationship between sarcopenia and the presence of kidney stones.

	Model 1	Model 2	Model 3	Model 4
	aOR (95% CI)	aOR (95% CI)	aOR (95% CI)	aOR (95% CI)
All participants				
Sarcopenia				
Yes	2.325 (1.602–3.376)	2.300 (1.572–3.366)	2.342 (1.588–3.455)	2.365 (1.598–3.500)
No	Reference	Reference	Reference	Reference
p for trend	<0.001	<0.001	<0.001	<0.001
Age <40 years				
Sarcopenia				
Yes	5.600 (2.287–13.710)	5.945 (2.362–14.964)	6.334 (2.479–16.180)	6.793 (2.619–17.618)
No	Reference	Reference	Reference	Reference
p for trend	<0.001	<0.001	<0.001	<0.001
Age ≥40 years				
Sarcopenia				
Yes	1.787 (1.173–2.723)	1.761 (1.148–2.700)	1.756 (1.132–2.723)	1.771 (1.138–2.757)
No	Reference	Reference	Reference	Reference
p for trend	0.007	0.010	0.012	0.011

Adjusted covariates: model 1: univariate analysis; model 2: gender, age and race; model 3: model 2 plus education level, marital status, and BMI; model 4: model 3 plus hypertension, smoking status, alcohol use, physical activities, blood urea nitrogen, creatinine, and uric acid. BMI, body mass index; aOR, adjusted odds ratio.

Data availability statement

Publicly available datasets were analyzed in this study. These data can be found here: National Health and Nutrition Examination Survey (NHANES).

Author contributions

BP, CX, and YW: conception and design. CT, HZ, and JN: administrative support. YW, YZ, and HS: provision of study materials or patients. TZ, YZ, and HZ: collection and assembly of data. CX, YZ, and HZ: data analysis and interpretation. All authors contributed to the article and approved the submitted version.

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Conflict of interest

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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Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2023.1123588/full#supplementary-material

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Prognostic value of sarcopenia in patients with lung cancer treated with epidermal growth factor receptor tyrosine kinase inhibitors or immune checkpoint inhibitors

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Objectives: It remains controversial whether sarcopenia has any significant impact on the efficacy of epidermal growth factor receptor tyrosine kinase inhibitors (EGFR-TKIs) or immune checkpoint inhibitors (ICIs) in patients with advanced non-small cell lung cancer (NSCLC). Therefore, in this study, we aimed to assess the association between sarcopenia and clinical outcomes in patients with advanced NSCLC receiving EGFR-TKIs or ICIs as a first-line therapy.

Methods: We retrospectively enrolled 131 patients with advanced NSCLC treated with first-line EGFR-TKIs or ICIs between 1 March 2019 and 31 March 2021. To estimate sarcopenia, we calculated skeletal muscle index (SMI) as the ratio of skeletal muscle area (cm^2) to height squared (m^2). Associations between sarcopenia and overall survival (OS) and progression-free survival (PFS) were evaluated using the Kaplan–Meier method and log-rank tests, respectively. A Cox proportional hazards regression model was used to assess the factors associated with OS and PFS. The Student's *t*-test or Mann–Whitney U test was used to compare the SMI between patients with or without objective response and disease control. The chi-squared test was used to compare adverse events (AEs) between patients with and without sarcopenia.

Results: Among the 131 patients, 35 (26.7%) were diagnosed with sarcopenia. Sarcopenia was an independent predictor of poor OS and PFS ($p < 0.05$) overall and in the EGFR-TKI- and ICI-treated cohorts. Among all patients, those with sarcopenia showed significantly shorter OS and PFS than those without sarcopenia (median OS and PFS: 13.0 vs. 26.0 months and 6.4 vs. 15.1 months; both $p < 0.001$). These associations were consistent across the subtypes of most clinical characteristics. Statistically significant differences between the objective response (OR) and non-OR groups were also observed in the mean SMI (OR group, 43.89 ± 7.55 vs. non-OR group, 38.84 ± 7.11 cm^2/m^2; $p < 0.001$). In addition, we observed similar results with disease control (DC) and non-DC

groups (DC group, 42.46 ± 7.64 vs. non-DCR group, 33.74 ± 4.31 cm^2/m^2; $p < 0.001$). The AEs did not differ significantly between the sarcopenia and non-sarcopenia groups.

Conclusion: Sarcopenia before treatment might be a significant predictor of poor clinical outcomes (shorter OS and PFS, fewer ORs, less DC) in patients with advanced NSCLC treated with EGFR-TKIs or ICIs as the first-line therapy.

KEYWORDS

sarcopenia, lung cancer, immune-checkpoint inhibitors, EGFR-TKIs, prognosis

1. Introduction

Lung cancer is the second most common malignant tumor and the leading cause of cancer-related deaths worldwide. Non-small cell lung cancer (NSCLC) comprises the majority (85%) of all lung cancers (1). Approximately, 25% of NSCLC patients present with an advanced stage at initial diagnosis (2), with a 5-year survival rate of less than 20% (3).

Epidermal growth factor receptor-tyrosine kinase inhibitors (EGFR-TKIs) and immune checkpoint inhibitors (ICIs) have been shown to significantly improve the survival of metastatic NSCLC patients with mutant and wild-type EGFR, respectively (4, 5). However, not all eligible patients can benefit equally from EGFR-TKIs or ICIs (6, 7). Although EGFR mutation and the PD-L1 expression level have been reported as potential predictors of the therapeutic efficacy for EGFR-TKIs and ICIs, it is essential to identify additional biomarkers that can help determine those patients most likely to benefit from these therapies.

Sarcopenia is characterized by progressive loss of skeletal muscle strength and mass and is associated with decreased muscle protein synthesis and increased protein degradation (8, 9). Sarcopenia has been reported in approximately 50% of lung cancer patients and is associated with a decrease in the efficacy of surgery or chemotherapy, toxicity, and a worse quality of life (10, 11). However, the impact of sarcopenia on the efficacy and toxicity of EGFR-TKIs and ICIs remains unclear.

Several articles have suggested that sarcopenia is correlated with poor clinical outcomes in patients receiving PD-1/PD-L1 inhibitors (12, 13), however, other researches have reached inconsistent conclusions (14–16). The same controversy also exists In NSCLC patients treated with EGFR-TKIs. A retrospective study showed that sarcopenia did not affect the response to gefitinib in patients with EGFR-mutated NSCLC (17). In contrast, another retrospective study enrolling 72 NSCLC patients treated with erlotinib found that sarcopenia was a negative biomarker that was significantly associated with response and survival outcomes (18).

In brief, there is no consensus as to whether sarcopenia is a prognostic biomarker for EGFR-TKI or ICI treatment of advanced NSCLC, especially when used as the first-line treatment. Consequently, we sought to investigate the potential predictive value of sarcopenia on the efficacy of EGFR-TKIs in NSCLC patients harboring EGFR mutations or of ICIs in patients with wild-type EGFR.

2. Materials and methods

2.1. Patients

In this study, we retrospectively collected the data of patients with pathologically confirmed metastatic NSCLC who were treated with EGFR-TKIs or ICIs as the first-line therapy at Sichuan Cancer Hospital, China, from 16 January 2018 to 8 June 2021. Patients who met the inclusion criteria below were enrolled: (1) histologically confirmed stage IV metastatic NSCLC; (2) treated with EGFR-TKIs or ICIs as first-line therapies; and (3) underwent a chest/abdominal CT scan within 4 weeks prior to EGFR-TKI or ICI therapy. This study was approved by the Ethics Committee of Sichuan Cancer Hospital and carried out in strict accordance with the Declaration of Helsinki.

2.2. Data collection

We consecutively enrolled 205 patients with advanced NSCLC treated with EGFR-TKIs or ICIs in our hospital. After selection according to the inclusion and exclusion criteria, 54 eligible patients receiving ICIs and 77 eligible patients receiving EGFR-TKIs were included (**Supplementary Figure 1**).

Subsequently, we obtained basic demographic and clinical data for all eligible patients, including age, sex, history of smoking and alcohol consumption, Karnofsky performance status (KPS) score, histopathology, height, weight, routine biochemical and hematological test results, CT images, EGFR mutation status, PD-L1 expression before EGFR-TKI or ICI initiation, treatment option, treatment response, and toxicity. The follow-up date ended at the date of the outcome event, the date of death, or the end of follow-up, whichever came first.

2.3. Skeletal muscle measurement and definition of sarcopenia

The chest/abdomen CT scan for sarcopenia evaluation was obtained within 4 weeks before the start of EGFR-TKIs or ICIs. The skeletal muscle area was measured at the L3 level by two experienced radiologists (NJY and XYN) using sliceOmatic

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TABLE 1 Baseline characteristics of all patients.

Characteristic	Non-sarcopenia	Sarcopenia	*p*-value
N	96(73.3%)	35(26.7%)	
Sex, *n* (%)			0.076
Female	44 (45.8%)	10 (28.6%)	
Male	52 (54.2%)	25 (71.4%)	
Smoking, *n* (%)			**0.038**
No	58 (60.4%)	14 (40%)	
Yes	38 (39.6%)	21 (60%)	
Drinking, *n* (%)			**0.041**
No	77 (80.2%)	22 (62.9%)	
Yes	19 (19.8%)	13 (37.1%)	
Histopathology, *n* (%)			0.764
AC	79 (82.3%)	28 (80%)	
SCC	17 (17.7%)	7 (20%)	
EGFR mutations, *n* (%)			0.152
No	36 (37.5%)	18 (51.4%)	
Yes	60 (62.5%)	17 (48.6%)	
EGFR mutation sites, *n* (%)			0.142
None	36 (37.5%)	18 (51.4%)	
Exon 19	34 (35.4%)	13 (37.1%)	
Exon 20	1 (1%)	1 (2.9%)	
Exon 21	25 (26%)	3 (8.6%)	
PD-L1 expression, *n* (%)			0.081
<1%	15 (15.6%)	7 (20%)	
1–50%	12 (12.5%)	10 (28.6%)	
>50%	10 (10.4%)	1 (2.9%)	
Unknown	59 (61.5%)	17 (48.6%)	
Chemotherapy, *n* (%)			0.237
No	52 (54.2%)	23 (65.7%)	
Yes	44 (45.8%)	12 (34.3%)	
EGFR-TKI therapy, *n* (%)			0.152
No	36 (37.5%)	18 (51.4%)	
Yes	60 (62.5%)	17 (48.6%)	
EGFR-TKI drugs, *n* (%)			0.176
None	36 (37.5%)	18 (51.4%)	
1st generation	26 (27.1%)	5 (14.3%)	
2nd generation	5 (5.2%)	0 (0%)	
3rd generation	29 (30.2%)	12 (34.3%)	
ICI therapy, *n* (%)			0.152
No	60 (62.5%)	17 (48.6%)	
Yes	36 (37.5%)	18 (51.4%)	
ICI drugs, *n* (%)			0.053
None	60 (62.5%)	17 (48.6%)	
PD-1	35 (36.5%)	15 (42.9%)	
PD-L1	1 (1%)	3 (8.6%)	
KPS score, *n* (%)			**0.002**
70	4 (4.2%)	6 (17.1%)	
80	49 (51%)	24 (68.6%)	
90	42 (43.8%)	4 (11.4%)	
100	1 (1%)	1 (2.9%)	
Age (years), mean ± SD	59.08 ± 9.93	59.09 ± 10.20	0.999
BMI, mean ± SD	22.91 ± 3.03	22.12 ± 2.97	0.188
Hemoglobin, mean ± SD	126.61 ± 18.04	122.71 ± 16.14	0.263
hCRP, median (IQR)	3.49 (0.77, 11.55)	6.77 (1.69, 22.07)	0.165
Total protein, mean ± SD	65.45 ± 6.31	64.06 ± 4.72	0.239
Albumin, mean ± SD	38.64 ± 4.85	36.54 ± 3.95	**0.023**

Bold values mean $p < 0.05$. SCC, squamous cell carcinoma; AC, adenocarcinoma; EGFR-TKI, epidermal growth factor receptor tyrosine kinase inhibitors; ICI, immune checkpoint inhibitor; PD-1, programmed death-1; PD-L1, programmed cell death-ligand 1; KPS, Karnofsky performance status; SD, standard deviation; BMI, body mass index; IQR, interquartile range; hCRP, hypersensitive C-reactive protein.

(TomoVision 5.0, Magog, QC, Canada) with −29 to 150 Hounsfield unit (HU) (**Supplementary Figure 2**). The skeletal muscle index (SMI) was calculated using the formula (L3 muscle area in cm^2)/(patient height in m^2). Sarcopenia was defined as a low SMI as follows: (1) for women, SMI < 31.6 cm^2/m^2; (2) for men, SMI < 40.2 cm^2/m^2 (19).

2.4. Follow-up

Tumor response evaluations were performed based on chest CT according to the Response Evaluation Criteria in Solid Tumours (RECIST) version 1.1 (for patients receiving EGFR-TKIs) or iRECIST criteria (for patients receiving ICIs). PFS and overall survival (OS) were calculated from the date of initiation of EGFR-TKI or ICI treatment to the date of progression (for PFS) or patient death (for OS) or to the last follow-up. The incidence and severity of all adverse events (AEs) were monitored and evaluated according to the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE V5.0).

2.5. Statistical analysis

We used R software, version 4.0.2 (R Foundation for Statistical Computing), for statistical analysis. Multiple Cox regression analyses were conducted to evaluate the impact of sarcopenia and other candidate prognostic factors on the OS and PFS. The Kaplan–Meier method and log-rank test were used to compare OS and PFS between patients with and without sarcopenia. The association between the presence of sarcopenia and demographic, clinical, and laboratory parameters, treatment response, and occurrence

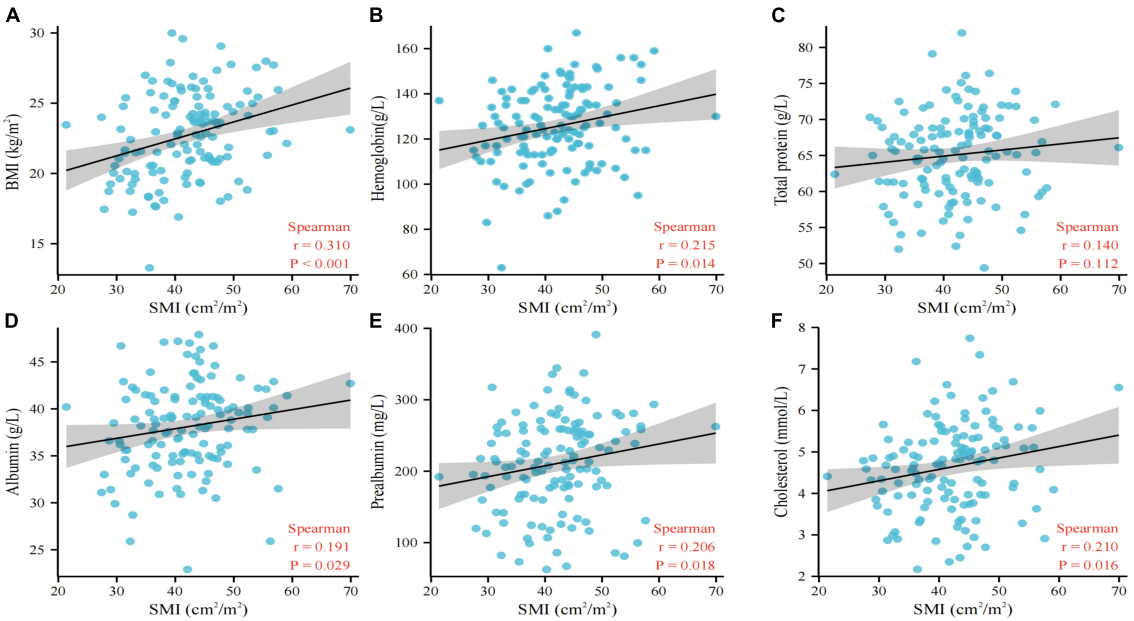

FIGURE 1
Association between BMI **(A)**, hemoglobin **(B)**, total protein **(C)**, albumin **(D)**, prealbumin **(E)**, cholesterol **(F)**, and L3 SMI. BMI, body mass index; SMI, skeletal muscle index.

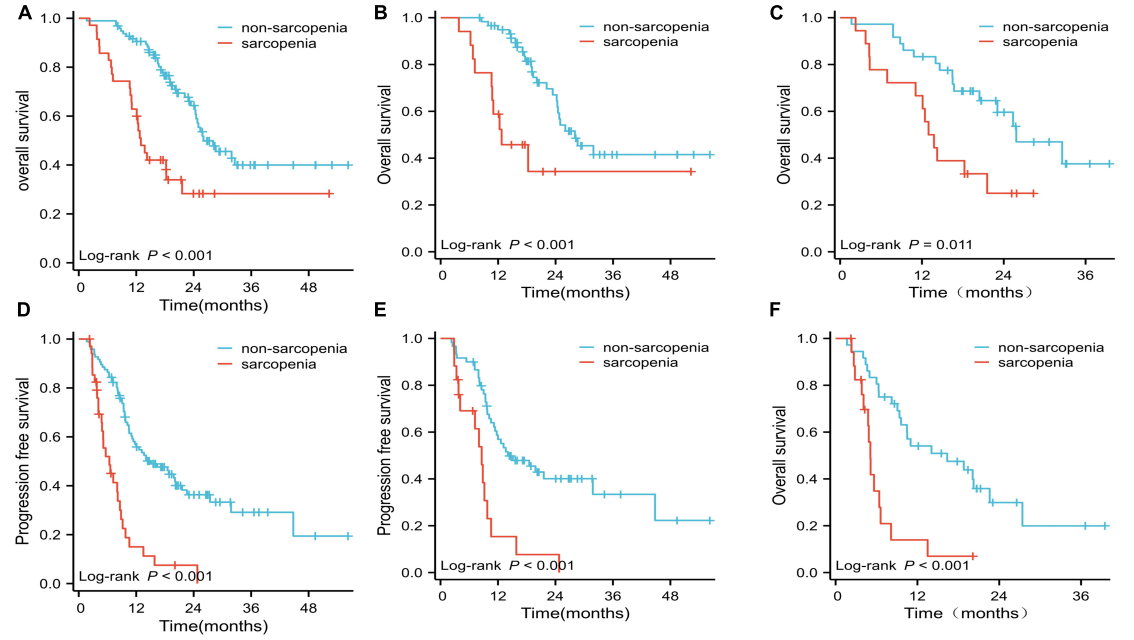

FIGURE 2
Overall survival (OS) and progression-free survival (PFS) curves. **(A)** OS for the two groups in the whole cohort; **(B)** OS for the two groups treated with EGFR-TKIs; **(C)** OS for the two groups treated with ICIs; **(D)** PFS for the sarcopenia and non-sarcopenia groups in the whole cohort; **(E)** PFS for the two groups treated with EGFR-TKIs; **(F)** PFS for the two groups treated with ICIs.

of AEs was established using the exact Fisher test and χ^2 test. Scatter plots were used to graphically represent the association between sarcopenia and hemoglobin, total protein, albumin, prealbumin, serum triglyceride, and serum cholesterol levels and BMI using Spearman's correlation. All statistics were two-tailed, and p-values ≤ 0.05 were considered statistically significant.

3. Results

3.1. Patient characteristics

The baseline characteristics of the 131 enrolled patients are presented in Table 1. A total of 77 and 54 patients received

first-line treatment with EGFR-TKIs or ICIs, respectively. Patients were categorized into two groups (sarcopenia and non-sarcopenia) according to the previously defined criteria for sarcopenia. Among all patients, 35 (26.7%) were diagnosed with sarcopenia, including 17 EGFR-TKI-treated and 18 ICI-treated patients. A full comparison of the baseline characteristics between the sarcopenia and non-sarcopenia groups is presented in Table 1. Sarcopenia was significantly more common in patients who had smoked ($p = 0.038$) or drunk ($p = 0.041$) regularly, or those who had a low KPS score ($p = 0.002$) or low albumin ($p = 0.023$). The baseline characteristics of the EGFR-TKI and ICI cohorts are listed in the Supplementary Tables 1, 2, respectively.

The relationship between nutritional status and L3 SMI is shown in Figure 1. The scatter plot shows a high correlation between nutritional status factors, including BMI ($p < 0.001$, Figure 1A), hemoglobin level ($p = 0.014$, Figure 1B), albumin level ($p = 0.029$, Figure 1D), prealbumin level ($p = 0.018$, Figure 1E), and cholesterol level ($p = 0.016$, Figure 1F), and L3 SMI, indicating that poor nutritional status is a risk factor for sarcopenia in NSCLC patients.

TABLE 2 Results of Cox regression analysis for overall survival for all patients.

Characteristics	Total (n)	Univariate analysis		Multivariate analysis	
		Hazard ratio (95% CI)	*p*-value	Hazard ratio (95% CI)	*p*-value
Sex	131				
Female	54	Reference			
Male	77	1.210 (0.729–2.010)	0.460		
Age	131	1.005 (0.980–1.031)	0.689		
Smoking	131				
No	72	Reference			
Yes	59	1.119 (0.684–1.830)	0.655		
Drinking	131				
No	99	Reference			
Yes	32	1.205 (0.691–2.101)	0.510		
Histopathology	131				
AC	107	Reference			
SCC	24	1.258 (0.668–2.370)	0.477		
Ch'emotherapy	131				
No	75	Reference			
Yes	56	1.070 (0.652–1.754)	0.789		
EGFR-TKIs therapy	131				
Yes	77	Reference			
No	54	1.389 (0.848–2.274)	0.192		
Body mass index	131	0.918 (0.844–1.000)	0.050	0.948 (0.866–1.038)	0.252
Sarcopenia status	131				
Non-sarcopenia	63	Reference		Reference	
Sarcopenia	68	2.940 (1.744–4.956)	**<0.001**	2.187 (1.230–3.891)	**0.008**
KPS score	131				
70	10	Reference		Reference	
80	73	0.423 (0.188–0.951)	**0.037**	0.437 (0.178–1.074)	0.071
90	46	0.180 (0.074–0.439)	**<0.001**	0.358 (0.128–1.001)	**0.050**
100	2	0.000 (0.000–Inf)	0.996	0.000 (0.000–Inf)	0.996
Hemoglobin	131	0.982 (0.969–0.995)	**0.005**	1.000 (0.983–1.018)	0.967
hCRP	131	1.010 (1.001–1.019)	**0.035**	1.004 (0.992–1.016)	0.538
Total protein	131	0.973 (0.936–1.012)	0.168		
Albumin	131	0.904 (0.864–0.946)	**<0.001**	0.921 (0.860–0.987)	**0.019**

Bold values mean $p < 0.05$. KPS, Karnofsky performance status; SCC, squamous cell carcinoma; AC, adenocarcinoma; hCRP, hypersensitive C-reactive protein; EGFR-TKIs, epidermal growth factor receptor-tyrosine kinase inhibitors.

3.2. Effect of sarcopenia on OS and PFS

The Kaplan–Meier curves for OS and PFS grouped by sarcopenia and non-sarcopenia are shown in Figure 2. Analysis of the entire patient cohort showed a significant difference in OS between patients with sarcopenia and those without sarcopenia, with a median OS of 13 and 26 months, respectively ($p < 0.001$; Figure 2A). Kaplan–Meier analysis also revealed that patients with sarcopenia had a significantly shorter PFS than those without sarcopenia (6.4 months vs. 15.1 months, $p < 0.001$; Figure 2D).

In the EGFR-TKI-treated cohort ($n = 77$), the median OS and PFS for patients with sarcopenia were significantly shorter than those for patients without sarcopenia (OS: 12.7 vs. 28.0 months; PFS: 8.6 vs. 14.1 months, respectively; both $p < 0.001$; Figures 2B, E). The median OS and PFS for patients with sarcopenia in the ICI-treated cohort were significantly shorter than those for patients without sarcopenia (OS: 13.4 vs. 25.8 months, $p = 0.011$; PFS: 5.1 vs. 16.4 months, $p < 0.001$; Figures 2C, F).

In the entire patient cohort, univariate Cox regression analysis revealed that BMI, sarcopenia, KPS score, levels of hemoglobin, hypersensitive C-reactive protein (hCRP) and albumin were significant prognostic factors for OS (all $p < 0.05$; Table 2). Multivariate analysis of all the above potential factors identified

TABLE 3 Results of Cox regression analysis for PFS for all patients.

Characteristics	Total (*n*)	Univariate analysis		Multivariate analysis	
		Hazard ratio (95% CI)	*p*-value	Hazard ratio (95% CI)	*p*-value
Sex	131				
Female	54	Reference			
Male	77	1.051 (0.683–1.616)	0.822		
Age	131	0.985 (0.964–1.007)	0.174		
Smoking	131				
No	72	Reference			
Yes	59	0.971 (0.631–1.493)	0.893		
Drinking	131				
No	99	Reference			
Yes	32	0.865 (0.514–1.455)	0.584		
Histopathology	131				
AC	107	Reference			
SCC	24	1.187 (0.689–2.047)	0.537		
Chemotherapy	131				
No	75	Reference			
Yes	56	1.042 (0.681–1.596)	0.849		
EGFR-TKIs therapy	131				
No	54	Reference			
Yes	77	1.372 (0.893–2.110)	0.149		
Body mass index	131	0.908 (0.846–0.974)	**0.007**	0.883 (0.814–0.958)	**0.003**
Sarcopenia status	131				
Non-sarcopenia	96	Reference		Reference	
Sarcopenia	35	3.590 (2.236–5.762)	<**0.001**	2.830 (1.662–4.817)	< **0.001**
KPS score	131		<**0.001**		
70	10	Reference		Reference	
80	73	0.977 (0.419–2.278)	0.958	1.638 (0.651–4.125)	0.295
90	46	0.281 (0.110–0.714)	**0.008**	0.696 (0.238–2.035)	0.508
100	2	0.000 (0.000–Inf)	0.995	0.000 (0.000–Inf)	0.996
Hemoglobin	131	0.989 (0.978–1.000)	0.055	1.002 (0.987–1.018)	0.773
hCRP	131	1.010 (1.001–1.018)	**0.022**	1.006 (0.994–1.018)	0.347
Total protein	131	0.971 (0.940–1.004)	0.085	0.991 (0.950–1.035)	0.689
Albumin	131	0.930 (0.893–0.968)	<**0.001**	0.977 (0.911–1.047)	0.508

Bold values mean $p < 0.05$. KPS, Karnofsky performance status; SCC, squamous cell carcinoma; AC, adenocarcinoma; EGFR-TKIs, epidermal growth factor receptor-tyrosine kinase inhibitors, hCRP, hypersensitive C-reactive protein.

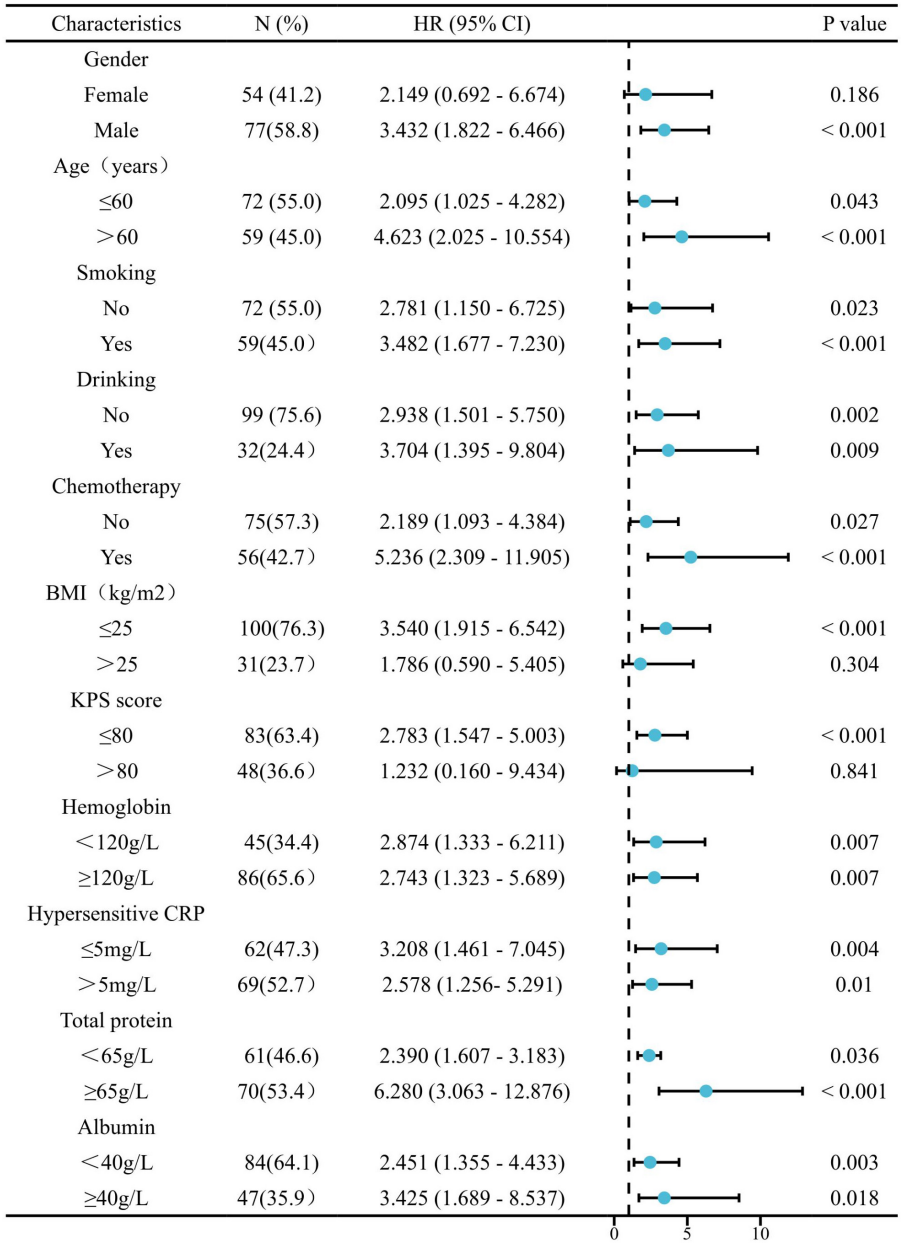

FIGURE 3
The association between sarcopenia and hazard ratios of OS in various subgroups. BMI, body mass index; KPS, Karnofsky performance status; hCRP, hypersensitive C-reactive protein.

only sarcopenia [hazard ratio (HR): 2.187, 95% confidence interval (CI): 1.230–3.891, $p = 0.008$; Table 2] and albumin [hazard ratio (HR): 0.921, 95% confidence interval (CI): 0.860–0.987, $p = 0.019$; Table 2] as strong independent predictors of OS. Univariate and multivariate Cox regression analyses of both the EGFR-TKI-treated and ICI-treated cohorts confirmed that sarcopenia was an independent negative factor for OS (EGFR-TKI-treated group, HR: 2.806, 95% CI: 1.304–6.037, $p = 0.008$; Supplementary Table 3; ICI-treated group, HR: 2.155, 95% CI: 1.107–4.484, $p = 0.028$; Supplementary Table 4).

Univariate Cox regression analysis revealed that BMI, sarcopenia, KPS score, hCRP level, and albumin level were significant prognostic factors for PFS (all $p < 0.05$; Table 3). Multivariate analysis confirmed the independent prognostic relevance of BMI (HR, 0.883; 95% CI: 0.814–0.958, $p = 0.003$) and sarcopenia (HR, 2.830; 95% CI: 1.662–4.817, $p < 0.001$) for PFS (Table 3). Univariate and multivariate Cox regression analyses of both the EGFR-TKI and ICI cohorts confirmed that sarcopenia was an independent negative factor for PFS (EGFR-TKI cohort, HR: 2.946, 95% CI: 1.430–6.068, $p = 0.003$, Supplementary Table 5; ICI cohort, HR: 3.567, 95% CI: 1.647–7.724, $p = 0.001$, Supplementary Table 6).

Stratified analyses were performed to clarify the relationship between sarcopenia and the HRs of OS and PFS in various patient subgroups (Figures 3, 4). Overall, sarcopenia was consistently associated with poor OS and PFS across most subgroups of patients.

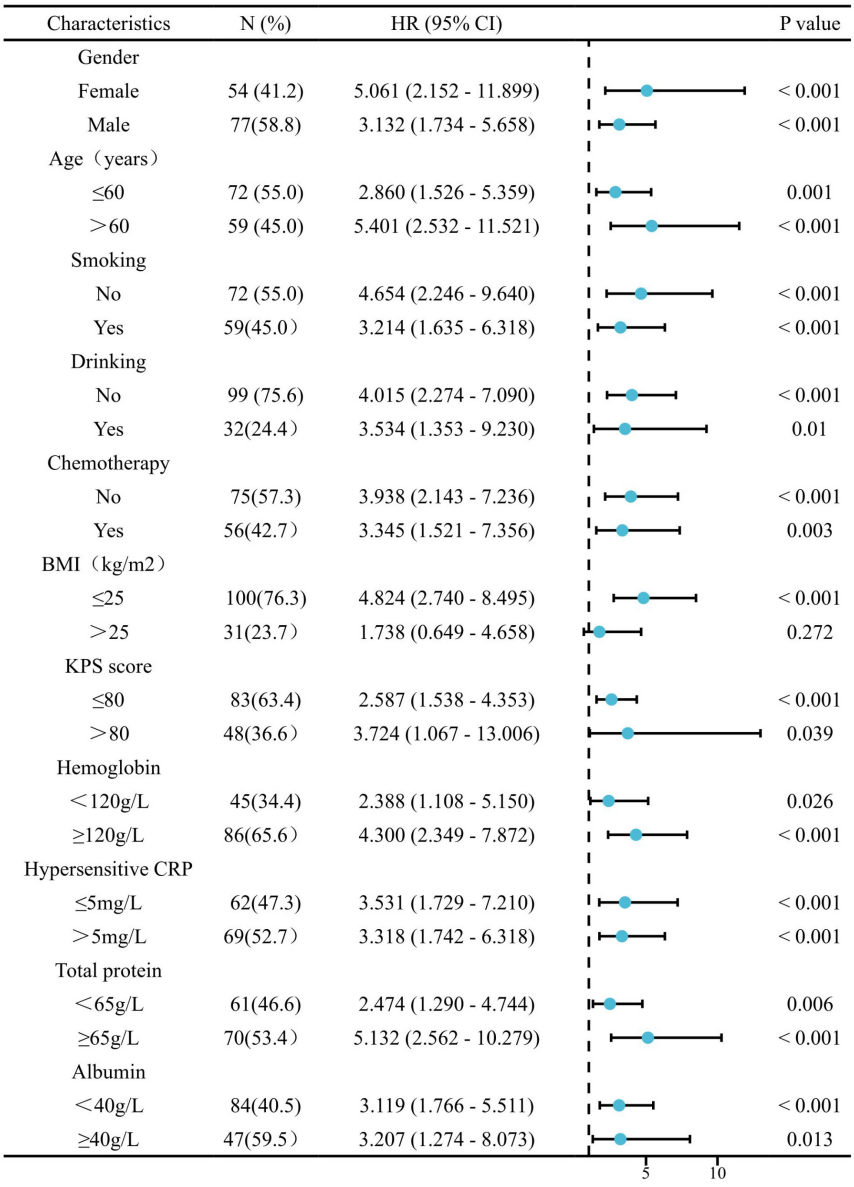

FIGURE 4
The association between sarcopenia and hazard ratios of PFS in various subgroups. BMI, body mass index; KPS, Karnofsky performance status; hCRP, hypersensitive C-reactive protein.

3.3. OR, DC, and treatment-related AEs

Of the 131 patients, 81 had an OR and 125 had DC. The mean SMI was significantly lower in the non-OR group than in the OR group, 38.84 ± 7.11 vs. 43.89 ± 7.55 cm^2/m^2, respectively ($p < 0.001$; Figure 5A). Similarly, a significant difference was also found in SMI between the DC group and non-DC group (42.46 ± 7.64 vs. 33.74 ± 4.31 cm^2/m^2, $p = 0.002$; Figure 5D). All analyses were repeated in the subgroups of patients treated with EGFR-TKIs or ICIs, and the findings were similar to those of the primary analysis (Figures 5B, C, E, F). The patients in the OR and DC groups had a significantly higher SMI than those in the non-OR and non-DC groups, regardless of whether the patients received EGFR-TKI (Figures 5B, E) or ICI (Figures 5C, F) treatment.

In our study, 51 patients (38.9%) experienced treatment-related AEs: 12 (12/35, 34.3%) in the sarcopenia group and 39 (39/96, 40.6%) in the non-sarcopenia group. There was no statistically significant difference between the two groups ($p = 0.550$). The most frequent AEs were hypothyroidism and skin rashes.

4. Discussion

The present study presents considerable real-world data on sarcopenia as a prognostic marker in patients with advanced NSCLC receiving first-line EGFR-TKIs or ICIs. We confirmed that patients with sarcopenia had significantly shorter OS and PFS than those without sarcopenia in the entire patient, EGFR-TKI-treated, and ICI-treated cohorts. In addition, statistically significant

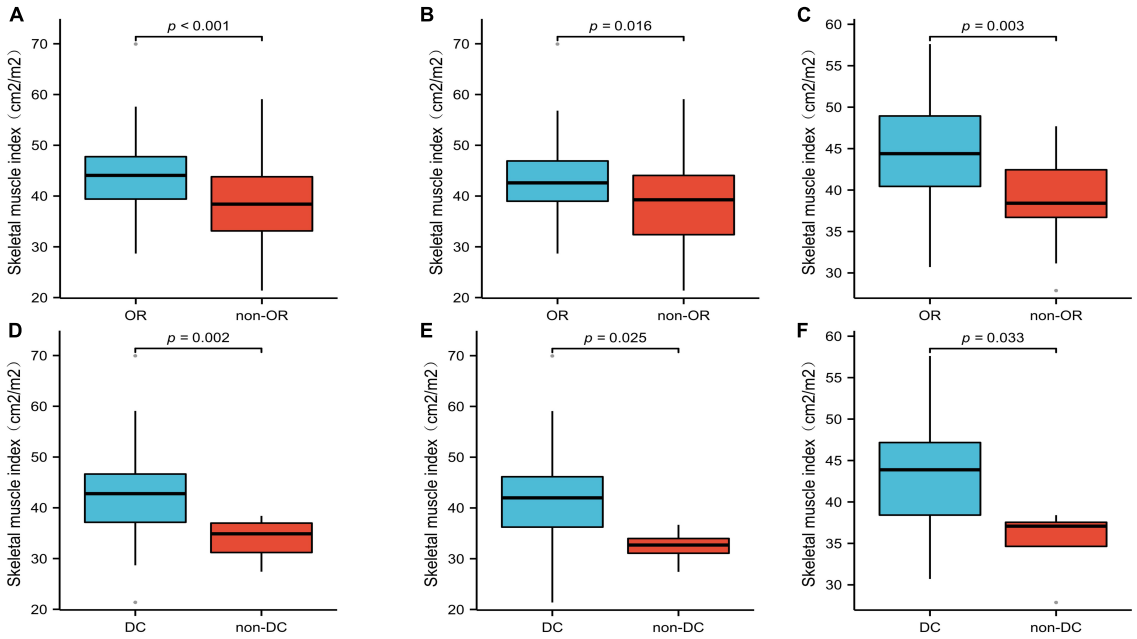

FIGURE 5
The mean SMI of the OR and DC groups in all patients **(A,D)**, patients treated with EGFR-TKIs **(B,E)**, and patients treated with ICIs **(C,F)**. SMI, skeletal muscle index; OR, objective response; DC, disease control.

differences were observed in mean SMI between the OR and non-OR groups and the DC and non-DC groups. Therefore, nutritional intervention and physical activity programs are recommended to patients with sarcopenia receiving immunotherapy or EGFR-TKI therapy to improve the therapy outcome.

Although there are many studies on the relationship between sarcopenia and immunotherapy in lung cancer, their conclusions are inconsistent. Most of these studies found that a low SMI or a sarcopenia diagnosis is associated with shortened survival in advanced NSCLC patients treated with PD1/PD-L1 checkpoint inhibitors (14, 16, 20–25). However, other studies showed no differences in OS and PFS between patients with and without sarcopenia (26–29). In these studies, ICIs were used in different treatment lines, which may have partially affected the results. The majority of patients included in these studies were treated with second-line or later immunotherapy, whereas only four studies enrolled patients who were receiving first-line immunotherapy, and the sample size in these was relatively small (16, 22, 23, 29). Currently, immunotherapy is increasingly used as the first-line treatment for advanced lung cancer; therefore, our study included advanced lung cancer patients receiving ICIs only as the first-line therapy. Our study included the largest sample size of patients receiving first-line immunotherapy reported to date and provided strong evidence of the negative impact of sarcopenia on the prognosis of lung cancer when using first-line ICIs.

Similarly, using univariate and multivariate analyses of EGFR-TKI subgroups, we found that patients without sarcopenia had significantly longer OS and PFS than those with sarcopenia. In the few studies evaluating the prognostic impact and predictive value of sarcopenia in NSCLC patients harboring EGFR mutations and treated with EGFR-TKIs, as with ICI therapies, the results are inconsistent; however, most studies agree that sarcopenia does not affect PFS and OS (30–32). In a retrospective study conducted by Sabrina et al., sarcopenia did not affect the response to gefitinib in patients with EGFR-mutated NSCLC, even though it was an indicator of poor prognosis in terms of OS (17). In contrast, Atakan et al. found that sarcopenia was an independent factor of poor prognosis for OS and PFS in NSCLC patients receiving EGFR-TKI-targeted therapy (18). These inconsistent results in different studies might come from different study design, different inclusion and exclusion criteria, different sample size and different way of measuring muscle area or definition of sarcopenia, etc.

Next, stratified analyses were performed to clarify the relationship between sarcopenia and the HRs of OS and PFS in various patient subgroups. Overall, sarcopenia was consistently associated with both poor OS and PFS across most subgroups of patients except for patients with BMI > 25kg/m^2. The reason may be that, for cancer patients, body weight and body fat are also important indicators to reflect the nutritional status of patients and significantly affect the treatment outcome of patients (33). Therefore, for obese (BMI > 25kg/m^2) cancer patients, a comprehensive body composition analysis may be a better prognostic indicator more than a single myopenia.

The underlying mechanisms by which sarcopenia affects the efficacy of ICIs and EGFR-TKIs are not yet fully understood. Previous studies have found that interleukin-15 is the most abundant cytokine expressed in the skeletal muscle that can regulate $CD8^+$ T cells and promote T cell survival (34, 35), which is important for maintaining immune function. Serum interleukin-15 levels decrease in older adults with the loss of muscle mass, suggesting that muscle loss may lead to impaired immune function, which may have some relevance to sarcopenia. Additionally, $CD4^+FoxP3^+$ Tregs infiltrate damaged skeletal muscles, suggesting that sarcopenia may play an important role in tumor immune

escape (36). Another possible mechanism for the poor prognosis of NSCLC patients with sarcopenia could be different drug clearance rates in cancer patients with or without sarcopenia, as there is a strong association between pembrolizumab clearance and OS. Patients with high ICI clearance rates had worse survival rates than those with low clearance rates. Some researchers believe the primary method of ICI elimination may be related to the development of cancer cachexia and sarcopenia. Procatabolic status can affect survival by leading to faster protein turnover through monoclonal antibody clearance (37).

The mechanism by which sarcopenia affects the efficacy of EGFR-TKIs is still unclear, but some of the reasons may be similar to those for immunotherapy drugs, such as drug clearance. Retrospective studies have shown that patients with the same body weight and BMI may have different skeletal muscle masses and adipose tissue levels, which could affect EGFR-TKI therapy outcomes (13). When administered, EGFR-TKIs, including gefitinib, are widely distributed in various tissues of the human body, and when bound to human serum albumin and α1-acid glycoprotein, they can have half-lives of up to 48 h. Researchers have demonstrated in animal models that gefitinib is present in lower concentrations in the skin and fat and in higher concentrations in highly perfused organs (38). In addition, studies have shown that gefitinib lasts for up to 96 h in muscle and for only 2 h in fat after oral consumption (39). Therefore, as the diffusion and disposition of drugs in fat are different from those in muscle, this could be one of the mechanisms by which sarcopenia affects the prognosis and toxicity of EGFR-TKIs.

Whether sarcopenia is associated with treatment-related toxicity in lung cancer remains unclear. In this study, we found that the treatment-related toxicities in patients with sarcopenic and non-sarcopenic lung cancer were similar (31, 34). Nie et al. reported that treatment-related toxicity occurred more frequently in patients with sarcopenic lung cancer using afatinib (30). In contrast, Alessio et al. did not find a significant relationship between baseline SMI and AEs (14). The toxicities of EGFR-TKIs or ICIs are closely related to the duration of medication. As the survival times of non-sarcopenia patients were longer than those of sarcopenia patients, this may have affected the incidence of adverse reactions, resulting in the lack of a statistically significant difference between the two groups.

Our study has several strengths. It is the first to include both EGFR-TKIs and ICIs, and the targeted immunotherapy included in our study was a first-line treatment, which conforms to the current standard treatment regimen. In addition, compared with similar studies, ours has the largest number of cases, and there are few studies focusing on both OS and PFS in patients, as in our study.

Our study also has several limitations. First, this was a retrospective, single-center study. Second, sarcopenia was defined only according to SMI and was not based on muscle strength and function, such as grip strength.

5. Conclusion

In conclusion, sarcopenia before first-line EGFR-TKI or ICI therapy might be a significant predictor of poor clinical outcomes, leading to shortened OS and PFS and reduced OR and DC. Sarcopenia should be considered before using EGFR-TKIs or ICIs in clinical practice.

Data availability statement

The original contributions presented in this study are included in the article/**Supplementary material**, further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving human participants were reviewed and approved by the Ethics Committee of Sichuan Cancer Hospital. The patients/participants provided their written informed consent to participate in this study.

Author contributions

JL and TL were responsible for conceptualizing and designing this study, data collection, data interpretation, and manuscript drafting. NY, LX, and XN played a major role in body composition assessment and data analysis. JX, YL, MZ, HZ, CT, SP, LL, HB, CL, and HK participated in acquisition of clinical records, data analysis, and revision of the manuscript. All authors read and approved the final version of manuscript.

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Conflict of interest

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.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2023.1113875/full#supplementary-material

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Sarcopenia is an independent risk factor for all-cause mortality rate in patients with diabetic foot ulcers

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Objective: This study aimed to determine whether sarcopenia affects the all-cause mortality rate of patients with diabetic foot ulcers (DFUs).

Research design and methods: The clinic-based observational study included 217 patients treated at the Department of Endocrinology, the First Affiliated Hospital of Chongqing Medical University during a 4-year period. All subjects underwent dual-energy X-ray absorptiometry to determine their body composition during hospitalization. Diagnosis of sarcopenia was based on the Baumgartner diagnostic criteria. Patients were followed up regularly by phone calls until April 1, 2019, and their survival status was recorded.Univariate and multivariate Cox risk ratio regression models were used to analyze factors influencing the all-cause mortality rate of patients with DFUs.

Results: Of the 217 patients, 158 people survived (82.7%), 33 died (17.3%), and 26 were lost to follow-up. The median follow-up time was 23 (Range 11–34) months. The majority of patients were male (68.6%), with a mean age of 67.29 ± 11.14 years. The 5-year survival rate was 68.3% and 45.9% for all study patients ($n = 217$) and sarcopenia patients ($n = 81$), respectively. Multivariate Cox risk regression model showed that age (HR 1.042[95%CI:1.006, 1.078], $P = 0.021$), sarcopenia (HR 5.051[95%CI:1.968, 12.961], $P = 0.001$), and serum creatinine (HR 1.007[95%CI: 1.003, 1.010], $P < 0.001$) were independent risk factors for all-cause mortality rate of patients with DFUs. Kaplan-Meier survival curve indicated that the survival rate of patients with sarcopenia was significantly lower than non-sarcopenia patients ($P < 0.001$).

Conclusions: Sarcopenia is an independent risk factor for all-cause mortality of patients with DFUs and hence an important prognostic factor for patients with DFUs. Active prevention and improvement of sarcopenia can potentially improve the survival outcomes of this patient population.

KEYWORDS

sarcopenia, diabetic foot ulcers, all-cause mortality, risk factor (RF), diabetes

1. Introduction

Diabetic foot ulcers (DFUs) represent a common, complex, and costly complication of diabetes. Current evidence suggests that advanced microangiopathy and macroangiopathy enact essential roles in the pathophysiology of DFUs, leading to high morbidity and mortality rates (1). The reported probability of developing DFUs is 25% (2, 3), while 5-year mortality of up to 40%has been documented (4). Ample evidence substantiates that risk factors, such as age, gender as male, peripheral vascular disease, kidney disease, major amputation, and low hemoglobin levels play an important role in mortality from DFUs (4–6). Our previous studies demonstrated that sarcopenia is an independent risk factor to DFUs. Interestingly, it has been reported that diabetics with sarcopenia are associated with a higher incidence of foot ulcers, Wagner grade, and amputation rate than those without sarcopenia (7).

According to the European Working Group on Sarcopenia in Older People (EWGSOP), sarcopenia is defined as a syndrome characterized by progressive and generalized loss of skeletal muscle mass and strength with a risk of adverse outcomes such as physical disability, poor quality of life, and death (8–10). In this regard, a prospective 7-year study demonstrated that sarcopenia increased mortality by 2.32 times (11). Indeed, sarcopenia is closely associated with diabetes, with reports suggesting that patients with type 2 diabetes harbor a higher risk of sarcopenia than those without diabetes (12). Hitherto, few studies have been conducted to explore the relationship between sarcopenia and mortality in the diabetic population. A study from South Korea reported that sarcopenia increased the risk of death in patients who underwent diabetic foot amputation (13). In fact, sarcopenia is emerging as a further severe complication in T2DM, in addition to those already well known, such as cardiovascular diseases (14). In T2DM, the core pathophysiologic defects are insulin resistance in the muscle and in the liver, and pancreatic beta-cell dysfunction. However, it has been recognized that other factors play a relevant role in T2DM, especially accelerated lipolysis, gastrointestinal incretin hormones deficiency/resistance, hyperglucagonemia, increased glucose reabsorption, and brain insulin resistance (15). Notably, these factors often present a common trait, that is, some degree of inflammatory condition. Inflammation, indeed, appears one of the factors which links T2DM and sarcopenia (16). Although diabetes has been reported to affect the prevalence of sarcopenia, no study has explored the association between sarcopenia and mortality in patients with diabetic foot ulcer. The present study explored the associations between sarcopenia and all-cause mortality from DFUs. We hypothesized that sarcopenia might be an independent risk factor in all-cause mortality from DFUs and a predictive factor for patient prognosis.

2. Research design and methods

2.1. Study design and population

This clinic-based observational study included a total of 217 patients with DFUs who visited the Diabetic Foot Multidisciplinary Team of the department of endocrinology, The First Affiliated Hospital of Chongqing Medical University hospital from January 2014 to September 2018 and voluntarily completed the body composition assessment. All patients received standardized treatment during hospitalization and were followed up every 2 years by phone calls after discharge to record their survival and wound healing situations until April 1, 2019, or death. Twenty-six patients were lost to follow-up, 33 patients died, and 158 patients survived at the end of follow-up. The survival information of 191 patients was collected. Written informed consent was obtained from each participant, and the study was approved by the Ethics Committee of The First Affiliated Hospital of Chongqing Medical University (approval number: 2020-238).

2.2. Clinical data collection

Clinical baseline data of the patients included demographic characteristics, duration of diabetes and duration of DFUs, hospitalization duration, amputation history, smoking habits, cardiovascular and cerebrovascular diseases, insulin therapy, and diabetic microvascular and macrovascular complications. The physical examination included an objective assessment of clinical symptoms of diabetic peripheral neuropathy and peripheral arterial disease (PAD). PAD was defined as an ankle-brachial pressure index (ABI) less than 0.9 with supporting imaging evidence by duplex ultrasonography or angiography. Laboratory examination included the white blood cell count, hemoglobin, percentage of neutrophils, albumin, triglycerides, total cholesterol, low-density lipoprotein cholesterol (LDL-c), high-density lipoprotein cholesterol (HDL-c), glycosylated hemoglobin (HbA1c), serum creatinine, serum uric acid, and urinary microalbumin to creatinine ratio (UACR). The body mass index (BMI) was calculated by dividing weight by the square of height (Kg/m2). HbA1c was measured using borate affinity high-performance liquid chromatography (Trinity Biotech, ultra, Dublin, Ireland). Serum lipids including total cholesterol, triglyceride, HDL-c, and LDL-c were measured enzymatically by an automatic analyzer (Model 7080; Hitachi, Tokyo, Japan) with reagents purchased from Leadman Biochemistry Co. Ltd. (Beijing, China). Serum creatinine, urinary creatinine, and albumin were measured by a fully automatic biochemical analyzer (Modular DDP, Roche). The urinary micro- albuminuria to creatinine ratio (UACR) was calculated.

2.3. Measurement of body components and diagnosis of sarcopenia

Body composition was measured using a DXA Hologic scanner (Hologic Discovery QDR® Series, Bedford, MA, USA) by a trained technician, including the fat and muscle mass in the head, limbs, trunk, and internal organs. All standard procedures were carried out as previously described in the literature. The Hologic Whole Body DXA reference database software was used to estimate the regional and whole-body lean tissue. The diagnostic criteria for sarcopenia were based on the Baumgartner diagnostic criteria: appendicular lean mass index (ALMI)=appendicular lean mass (ALM=Arm LM + Leg LM) / height2 in kg/m2. The diagnostic

criteria of sarcopenia were $ALMI < 7.01kg/m2$ and $< 5.42kg/m2$ in males and females, respectively (17).

2.4. Diagnosis and evaluation of diabetes and its complications

T2DM was diagnosed according to the diagnostic criteria for diabetes established by the World Health Organization (WHO) in 1999 (18). Chronic diabetic complications such as diabetic peripheral neuropathy, diabetic nephropathy, diabetic retinopathy, and diabetic foot disease were diagnosed using the 2012 American Diabetes Association (ADA) guidelines (18). The degree of diabetic peripheral neuropathy was assessed by the neuropathy symptom score (NSS) and neuropathy disability score (NDS). NSS was evaluated by asking patients about their experience of pain or discomfort in the legs. NDS was assessed by the Achilles reflex, vibratory sensation, temperature (cold tuning fork) sensation, 10 g monofilament proprioception, and pin-prick sensation (19). Nerve conduction velocity (NCV) was measured by EMG/Evoked Potentiometer (Type of Keypoint 9033A07, Dantec, Denmark). In addition, minor amputation was defined as amputation at the ankle joint level and below, while major amputation was defined as above the ankle joint.

3. Statistical analysis

Analyses were performed using SPSS 20.0 statistical software. Data were tested for normality and homogeneity of variance by a one-sample Kolmogorov-Smirnov test. Continuous variables that met the normal distribution were expressed as mean ± standard deviation. Independent samples *t*-test was used for comparison between two groups. Continuous variables that did not follow the normal distribution after data transformation were expressed as median (quartile). The comparison between groups was conducted using the two-sample Kolmogorov-Smirnov test. Categorical variables were expressed as frequencies and percentages, and the chi-square test was used for two or more groups of categorical variables. Kaplan-Meier survival curves were plotted, and the log-rank test was used to compare the survival of each group. Cox proportional hazards regression was used to obtain hazard ratio (HR) and 95% confidence interval (CI) of mortality. ăCovariates established as clinically significant predictors of death and with a $p \leq 0.10$ during univariate analysis were entered as covariates into multivariate proportional hazards regression models for death. A $P < 0.05$ was statistically significant.

4. Result

4.1. Baseline clinical and biochemical characteristics of the study population

Table 1 shows the baseline clinical data and biochemical tests of the survival or death groups of patients with DFUs. A total of 217 individuals were included in the study, consisting predominantly of males (68.6%) with a mean age of 67.29 ± 11.14 years. The survival status of patients was followed up by telephone every 2 years, with a median follow-up time of 23 (11–34) months. As of April 1, 2019, 26 patients were lost to follow-up. Survival data were available for 191 patients, of which 158 (82.7%) survived, and 33 (17.3%) patients died. As shown in Table 1, the death group was significantly older than the survival group ($P < 0.001$). Moreover, in the death group, the serum creatinine ($P = 0.001$) was significantly higher, and with a more significant proportion of patients with a previous history of foot ulcer ($P = 0.020$) and PAD ($P = 0.001$) compared to the survival group. Hemoglobin was significantly lower in the death group than in the survival group ($P = 0.022$). Importantly, the prevalence of sarcopenia was in the death group was significantly higher than in the survival group (78.8% vs. 34.8%, $P < 0.001$). No patients included in this study underwent major amputations, and no significant difference in the history of minor amputations was found between the two groups. In addition, gender, duration of diabetes, duration of diabetic foot ulcer, smoking habits, hypertension, coronary heart disease, and history of cerebrovascular disease exhibited no significant difference between the two groups.

4.2. Cumulative survival rates at 1, 3, and 5 years of follow-up for patients with diabetic foot ulcers

The cumulative survival rates of all patients at 1, 3, and 5 years of follow-up are presented in Table 2. The cumulative survival rate of diabetic foot ulcer patients with sarcopenia was 83.7% (75.3–92.1%) at 1 year and 45.9% (27.5–64.3%) at 5 years. Moreover, the cumulative survival rates at 1, 3, and 5 years were higher in patients with diabetic foot ulcers without sarcopenia than in patients with sarcopenia.

4.3. Prognostic factors for all-cause mortality rate in patients with DFUs (univariate and multivariate analyses)

Table 3 shows the Cox risk ratio model results of all-cause mortality during univariate and multivariate analysis in patients with DFUs. During univariate analysis, factors associated with higher mortality encompassed age, sarcopenia, hemoglobin, serum creatinine, and peripheral artery disease. Gender, hospitalization time, smoking, minor amputation, previous diabetes foot ulcers, Duration of Diabetes and DFUs, HbA1c, UACR, inflammatory markers level, blood lipids, insulin use, diabetic peripheral neuropathy, diabetic nephropathy, and diabetic retinopathy (partial data not shown) exhibited no significant associations with all-cause mortality of patients with DFUs. Indexes with a $P < 0.1$ in Table 1 were included in Model 1 during multivariate analysis. We found that age, sarcopenia, hemoglobin, and serum creatinine were correlated with all-cause mortality of patients with DFUs. ăGiven the clinical impact of chronic diseases on all-cause mortality, gender, hypertension, coronary heart disease, cerebrovascular disease, diabetic nephropathy, and statistically significant indicators from Model 1 were included in Model 2

TABLE 1 Baseline clinical and biochemical characteristics of the survival and death group of patients with diabetic foot ulcers.

	Alive (158,82.7%)	Death (33,17.3%)	*p*-value
Gender (male/female)	107/51	24/9	0.694
Age (year)	65.68 ± 10.69	75.00 ± 10.12	<0.001
The hospitalization time (days)	17 (11,24)	15 (11,20)	0.240
Duration of diabetes (year)	10 (5,17)	10 (5,16)	0.985
Duration of DFUs (year)	1.0 (0.5,3.0)	0.9 (0.3,2.7)	0.206
Smoking (%)	50.6	60.6	0.297
History of foot ulcer (%)	25.3	45.5	0.020
Previous minor amputation (%)	22.8	30.3	0.358
Sarcopenia (%)	34.8	78.8	<0.001
Hypertention (%)	61.4	57.6	0.683
Coronary heart disease (%)	18.4	27.3	0.243
Cerebrovascular disease (%)	15.8	21.3	0.451
BMI (Kg/m2)	24.55 ± 3.38	22.77 ± 3.89	0.101
White blood cell (*109/L)	8.41 ± 3.88	9.28 ± 4.61	0.262
Percentage of neutrophils (%)	71.86 ± 9.49	74.15 ± 10.59	0.220
Hemoglobin (g/L)	120.93 ± 18.58	112.39 ± 22.38	0.022
Albumin(g/L)	37 (33,41)	37 (32,39)	0.749
Total Cholesterol (mmol/L)	3.78 ± 1.08	3.58 ± 1.23	0.326
Triglyceride (mmol/L)	1.44 ± 1.51	1.23 ± 0.55	0.407
HDL-cholesterol (mmol/L)	1.04 (0.81, 1.21)	1.04 (0.86,1.23)	0.905
LDL-cholesterol (mmol/L)	2.19 (1.74, 2.94)	2.06 (1.45, 2.63)	0.446
Creatinine (umol/L)	73 (60,98)	91 (79, 157)	0.001
Uric acid (umol/L)	286.33 ± 104.25	297.67 ± 112.50	0.576
UACR (mg/g)	339.27 ± 658.01	408.71 ± 935.53	0.475
HbA1c (%)	9.87 ± 2.73	8.94 ± 2.28	0.081
ABI	1.15 (0.98 1.21)	0.88 (0.74, 1.10)	<0.001
Insulin use (%)	53.2	54.5	0.885
Chronic diabetic complications			
Diabetic peripheral neuropathy (%)	94.3	87.9	0.183
Diabetic kidney disease (%)	50.0	51.5	0.874
Diabetic retinopathy (%)	40.1	37.5	0.782
Peripheral artery disease (%)	19.7	52.2	0.001

DFU is associated with Wagner grading.

TABLE 2 Cumulative probabilities (with 95% CI) of survival.

	Year 1	Year 3	Year 5
All patients	92.1% (88.2–96.0%)	77.4% (69.4–85.4%)	68.3% (57.3–79.3%)
Patients with sarcopenia	83.7% (75.3–92.1%)	61.2% (46.9–75.5%)	45.9% (27.5–64.3%)
Patients without sarcopenia	98.2% (95.7–100.0%)	89.4% (80.8–98.0%)	85.4% (74.0–96.8%)

during multivariate analysis. We found that age (HR 1.042[95% CI: 1.006, 1.078], $P = 0.021$), sarcopenia (HR 5.051[95% CI: 1.968, 12.961], $P = 0.001$), and serum creatinine (HR 1.007[95% CI: 1.006, 1.078], $P = 0.021$). ă1.003, 1.010], $P < 0.001$) remained significantly correlated to all-cause mortality (Table 3). Kaplan-Meier survival curves of DFUs patients in Figure 1 with and without

TABLE 3 Prognostic factors for all-cause mortality rate in patients with DFUs (univariate and multivariate analyses).

Prognostic factor	Univariate		Multivariate (Model 1)		Multivariate (Model 2)	
	P-value	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value	HR (95% CI)
Age	<0.001	1.062 (1.029, 1.096)	0.026	1.064 (1.007, 1.123)	0.021	1.042 (1.006, 1.078)
History of foot ulcer	0.153	1.657 (0.829,3.309)	0.781	0.864 (0.309, 2.421)	-	-
Sarcopenia	<0.001	6.106 (2.646, 14.091)	0.003	5.411 (1.803, 16.242)	0.001	5.051 (1.968, 12.961)
Hemoglobin	0.016	0.979 (0.962, 0.996)	0.042	0.972 (0.945, 0.999)	0.242	0.988 (0.967, 1.008)
Creatinine	<0.001	1.006 (1.004, 1.009)	<0.001	1.007 (1.003, 1.010)	<0.001	1.007 (1.003, 1.010)
HbA1c	0.109	0.885 (0.761, 1.028)	0.512	1.069 (0.876, 1.304)	-	-
Peripheral artery disease	0.002	3.536 (1.559, 8.020)	0.914	0.942 (0.321, 2.770)	-	-

Notes:Model 1: Adjusted for age, history of foot ulcer, sarcopenia, hemoglobin, creatinine, HbA1c and peripheral artery disease; Model 2: Adjusted for age, sarcopenia, hemoglobin, creatinine, gender, hypertension, coronary heart disease, cerebrovascular disease and diabetic nephropathy. Abbreviations:HR, Hazard Ratio; 95% CI, 95% confidence intervals in brackets; HbA1c, glycosylated hemoglobin.

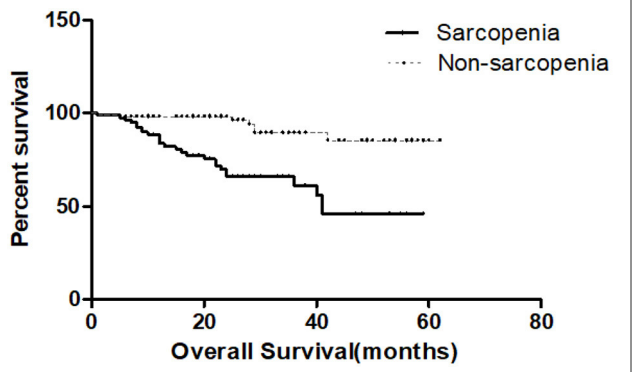

FIGURE 1
Kaplan-Meier survival plot for all subjects.

sarcopenia showed that the survival rate of DFUs patients with sarcopenia was lower than those without sarcopenia ($P < 0.001$).

4.4. Prevalence of chronic diseases among DFUs patients with and without sarcopenia

As shown in Table 4, gender ($P = 0.003$) and age ($P = 0.006$) exhibited statistically significant differences between the sarcopenia and non-sarcopenia groups. The proportion of males with sarcopenia was significantly higher. Moreover, the mortality rate in the sarcopenia group was significantly higher than in the non-sarcopenia group (32.1% vs. 6.4%, $P < 0.001$). It is worth noting that the prevalence rate of chronic diseases was significantly higher in the sarcopenia group, where the prevalence rates of cerebrovascular disease, coronary heart disease, peripheral artery disease, and diabetic nephropathy were higher than in the non-sarcopenia group.

5. Discussion

In this clinic-based study on the long-term outcomes of patients with DFUs, an association between sarcopenia and all-cause mortality was documented. To the best of our knowledge, few studies have investigated the association between sarcopenia and mortality in patients with DFUs. Importantly, this study showed that sarcopenia was an independent risk factor for all-cause mortality in DFUs patients, suggesting that sarcopenia may be a significant predictor of prognosis in this patient population. Aggressive prevention and improvement of sarcopenia may improve survival in this patient population.

The molecular mechanism of sarcopenia associated with DFD has not been thoroughly investigated. According to previous reports, several possible mechanisms could explain this association. First, skeletal muscle is considered to be an endocrine organ. Muscle factors and muscle metabolites secreted by skeletal muscle mediate the communication between muscles and other organs (20). Patients with sarcopenia have altered myocyte production in their muscles (20), which may be related to sarcopenia and DFD. Second, muscle weakness is associated with a higher risk of foot injury (21), which is a common cause of DFD. Third, myopenia and DFD share similar underlying mechanisms, including oxidative stress, chronic inflammation, and mitochondrial dysfunction (22–24). Muscle overproduction of reactive oxygen and nitrogen species is observed in sarcopenia, and the risk of sarcopenia is greatly reduced by specific inhibition of oxidative stress by muscle (25, 26). Observational and biopsy studies have strengthened the link between chronic low-grade inflammation and muscular atrophy (27). Mitochondrial dysfunction in skeletal muscle has been implicated in the pathogenesis of sarcopenia, and improving the quality control of mitochondria has been considered as a potential intervention for the management of sarcopenia11. In addition, the regenerative capacity of skeletal muscle is reduced in sarcopenia, andthe decline in stem cell regeneration is well recognized in sarcopenia (28, 29). Overproduction of reactive oxygen species and nitrogen species in sarcopenia may mediate the progression of neuropathy and vasculopathy and correlate sarcopenia with DFD. The main strength of our study is the relatively large sample size of DXA-based body composition measurements. Since sarcopenia is associated with DFD and DFD patients with sarcopenia have a poor prognosis.

An increasing body of evidence suggests that DFUs represent a marker of high mortality in diabetic patients (30–32). Walsh JW et al. reported a 5-year mortality rate of approximately 50% in

TABLE 4 Chronic disease in DFU patients with and without sarcopenia.

	Alive (81, 42.4%)	Death (110, 57.6%)	*p*-value
Gender (male/female)	65/16	66/44	0.003
Age (year)	69.86 ± 11.23	65.39 ± 10.73	0.006
Death (%)	32.1	6.4	<0.001
History of foot ulcer (%)	32.1	26.4	0.387
Previous minor amputation (%)	25.9	22.7	0.609
Hypertension (%)	66.7	56.4	0.150
Cerebrovascular disease (%)	24.7	10.9	0.012
Coronary heart disease (%)	27.2	14.5	0.031
Peripheral artery disease (%)	52.2	19.7	0.001
Diabetic Kidney Disease (%)	60.5	42.7	0.015

patients who developed DFUs (33). The overall 5-year survival rate of all patients with DFUs in this study was 68.3% and 45.9% for sarcopenia patients with DFUs. Serum creatinine, sarcopenia, and age were independent predictors of mortality in patients with DFUs after adjusting for multiple confounding factors. Hemoglobin and peripheral arterial disease were associated with DFU mortality during univariate analysis. A low hemoglobin often reflects a poor nutritional status and may be related to a poor patient prognosis. Moreover, it has been reported that peripheral vascular disease is a predictor of death for patients with DFU (34). However, the above findings were not observed after adjusting for confounding factors in this study. In addition, we found that serum creatinine is an independent risk factor for all-cause mortality of patients with DFUs. The degree of kidney damage has been documented to be closely related to the incidence and prevalence of DFU (35). In this respect, Wolf et al. reported that impaired renal function is an independent predictor of all-cause mortality and cardiovascular mortality (36). Moreover, lower limb amputation has been strongly associated with DFUs mortality and is reportedly an independent predictor of death (5, 37). There was no correlation between amputation and all-cause mortality of DFUs in this study, which may be attributed to the small number of DFUs patients with a history of amputation included in this study, and all amputation cases were minor.

It is well-established that sarcopenia is a strong predictive factor of all-cause mortality among community seniors (38, 39), nursing home residents (40), and hospitalized seniors (41). Interestingly, Atkins et al. reported that sarcopenia might be associated with cardiovascular mortality (42). Another long-term follow-up study of 15,000 Chinese middle-aged and elderly people showed that compared with normal people, the incidence of cardiovascular disease in middle-aged and elderly people with sarcopenia increased by 72%, and the risk of cardiovascular events increased by 33% (43). Consistently, the present study indicated that sarcopenia was an independent risk factor for all-cause mortality in patients with DFUs. As shown in Table 4, significant differences in gender and age were found between sarcopenia and non-sarcopenia patients. Importantly, the prevalence rates of diabetic nephropathy, peripheral artery disease, coronary heart disease, and cerebrovascular disease were higher among patients with sarcopenia, accounting for the significant impact of sarcopenia on mortality of patients with DFUs.

Several limitations and shortcomings were found in this study. First of all, only patients with DFUs who voluntarily completed the body composition exam were included, representing a source of selection bias. For clinical reasons, some severe patients with DFUs who were unable to move freely failed to complete body composition tests during hospitalization, but clinical observations found that these patients often had sarcopenia, which may allow us to underestimate the prevalence of sarcopenia in patients with DFUs. This is not conducive to our discovery of a possible closer association between sarcopenia and all-cause mortality in patients with DFUs. Moreover, the sample size of this study was relatively small, and the median follow-up time was short, which may lead to biased results. In addition, all amputation cases in this study were minor, which may be attributed to the fact that patients with major amputations exhibit poor ambulation and cannot complete the examination's physical component. Accordingly, we could not properly explore the relationship between mortality and major and minor amputations in sarcopenia patients. For patients lost to follow-up, it is highly likely that some patients were already deceased and could be contacted, which led to an underestimation of the actual mortality rate. Moreover, the specific cause of death could not be ascertained during telephone follow-up. Accordingly, this study only explored the association between sarcopenia and all-cause mortality of patients with DFUs. In addition, no data was available on muscle strength and muscle function, parameters emphasized in the diagnostic criteria of sarcopenia in recent years. Therefore, prospective follow-up studies with a larger sample size are warranted to increase the robustness of our findings and explore the associations between muscle strength and function and prognosis of this patient population.

In summary, sarcopenia is an independent risk factor for all-cause mortality of patients with DFUs, suggesting it is an important prognostic factor. Active prevention and improvement of sarcopenia may increase the survival rates of this patient population.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding authors.

Ethics statement

The studies involving human participants were reviewed and approved by the Ethics Committee of the First Affiliated Hospital of Chongqing Medical University (approval number: 2020-238). The patients/participants provided their written informed consent to participate in this study.

Author contributions

QC and QY designed the study. YZ, BZ, QZ, CY, JS, CZ, XN, and JC collected and collated clinical data. JH and YJ take responsibility for the accuracy of the data analysis. QY and XN drafted the manuscript. QC was the guarantor of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. All authors provided support for the analysis and interpretation of results, critically revised the manuscript, and approved the final manuscript.

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Conflict of interest

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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Measurement of sarcopenia in lung cancer inpatients and its association with frailty, nutritional risk, and malnutrition

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Background: Sarcopenia, frailty, and malnutrition are associated with undesirable clinical outcomes in cancer patients. Sarcopenia-related measurements may be promising fast biomarkers for frailty. Our objectives were to assess the prevalence of nutritional risk, malnutrition, frailty, and sarcopenia in lung cancer inpatients, and describe the relationship of them.

Methods: Stage III and IV lung cancer inpatients were recruited before chemotherapy. The skeletal muscle index (SMI) was assessed by multi-frequency bioelectric impedance analysis (m-BIA). Sarcopenia, frailty, nutritional risk, and malnutrition were diagnosed according to the Asian Working Group for Sarcopenia 2019 (AWGS 2019), Fried Frailty Phenotype (FFP), nutritional risk screening-2002 (NRS-2002), and Global Leadership Initiative on Malnutrition criteria (GLIM), and correlation analysis was performed between them with Pearson's *r* correlation coefficients. A univariate and multivariate logistic regression analysis was conducted for all patients, gender and age-stratified subgroups to obtain odds ratios (ORs) and 95% confidence intervals (95%CIs).

Results: The cohort included 97 men (77%) and 29 women (23%), with mean age of 64.8±8.7years. Among the 126 patients, 32 (25.4%) and 41 (32.5%) had sarcopenia and frailty, and the prevalence of nutritional risk and malnutrition was 31.0% (n =39) and 25.4% (n =32). Adjusted for age and gender, SMI was correlated with FFP (r =−0.204, p =0.027), and did not remain significantly when stratified by gender. Stratification according to age revealed in ≥65-years-old population, SMI and FFP were significantly correlated (r =−0.297, p =0.016), which is not seen in <65-years-old group (r =0.048, p =0.748). The multivariate regression analysis showed FFP, BMI, and ECOG were the independent variables associated with sarcopenia (OR 1.536, 95%CI 1.062–2.452, p =0.042; OR 0.625, 95%CI 0.479–0.815, p =0.001; OR 7.286, 95%CI 1.779–29.838, p =0.004).

Conclusion: Comprehensively assessed sarcopenia is independently associated with frailty based on FFP questionnaire, BMI, and ECOG. Therefore, sarcopenia assessment including m-BIA based SMI, and muscle strength and function could be used to indicate frailty to help select the targeting patients for care. Moreover, in addition to muscle mass, muscle quality should not be ignored in clinical practice.

KEYWORDS

sarcopenia, frailty, nutritional risk, malnutrition, lung cancer

Introduction

Lung cancer is one of the most common malignancies with the highest number of new cases and the highest mortality rate in China (1). Chemotherapy, radiotherapy, and immunotherapy are the main treatment modalities that improve patient survival and quality of life (2). Among all cancer types, lung cancer has the third highest malnutrition rate at 38% (3–5). As the realization of the importance of muscle, reduced skeletal muscle mass could be a marker for malnutrition, and is proven to be associated with increased incidence of antineoplastic therapy-induced toxicity, decreased survival, and poor clinical outcomes in cancer patients (6–10). It has been found the cumulative recurrence rate at 5 years after surgery was significantly higher in NSCLC patients with sarcopenia than in patients without sarcopenia (49.9 and 22.4%, respectively), suggesting sarcopenia (OR 2.52, $p = 0.001$) an independent risk factor for postoperative recurrence (11). Under the effect of the disease, unreasonable diet, and reduced activity, there may be fat gain, masking the decline in muscle and weight, so monitoring weight alone cannot adequately reflect the nutritional risk. Instead, early identification of sarcopenia makes it possible to detect nutritional risk earlier and conduct an intervention to reduce chemo-and radio-therapy toxicity and improve clinical outcomes.

Many prior studies retrospectively measured skeletal muscle area (SMA) at the L3 level of abdominal CT scan as a mean of assessing sarcopenia (6, 9, 10), however, the diagnosis of sarcopenia in either European Working Group on Sarcopenia in Older People (EWGSOP) (12) or Asian Working Group for Sarcopenia 2019 (AWGS 2019) (13) includes a comprehensive assessment of muscle mass, strength, and function, so imaging alone does not constitute a completed diagnostic element. In addition, there is a lack of universally accepted thresholds for SMI due to sample size and different ethnicities, so the SMI cut-off values published in the previous literature are often based on the lowest quartile of the target population.

Nutritional risk screening is used to find patients who may be at nutritional risk and to perform subsequent nutritional care. Recently, GLIM criteria have been used to evaluate malnutrition in oncology patients. The prevalence of malnutrition diagnosed according to GLIM in lung cancer patients is as high as 47.5%, and there is a significant relationship between malnutrition with early cessation of anti-cancer therapy, mortality, and quality of life (14). Moreover, neutrophil-to-lymphocyte ratio (NLR) is widely used as a prognostic marker for inflammation, progression free survival (PFS), and overall survival (OS) in cancers (15–17).

Both the nutrition risk screening-2002 (NRS-2002) and the GLIM malnutrition assessment focus on recent weight loss, reduced food intake, low BMI, and disease burden, whereas GLIM also includes an assessment of muscle mass. Frailty refers to the patient's vulnerability to the environment, and together with sarcopenia, it also involves a decrease in muscle strength and function. In addition, frailty focuses on the patients' subjective perception of fatigue, whereas sarcopenia pays more attention to the objective assessment of muscle mass, however, both of which neither consider the recent decline in dietary intake nor the burden of disease aspects. Many previous studies have focused on nutrition-related assessments in lung cancer patients, but there is a lacking of comprehensive assessment of above indicators and their interrelationships. Moreover, there is a lack of an optimized and brief nutritional assessment process concerning nutritional risk, frailty, and muscle status for lung cancer inpatients. Therefore, this study is to investigate the prevalence of sarcopenia in pre-treatment lung cancer inpatients and analyzes its association with frailty and other related factors. Nevertheless, we also explore the relationship between sarcopenia, nutritional risk, malnutrition, and frailty.

Materials and methods

Subjects

This study was performed in Peking Union Medical College Hospital between December 2021 and March 2022. The inclusion criteria for this prospective study were as follows: (1) ≥18 years of age without gender limitation, (2) radiologically or pathologically diagnosed stage III-IV lung cancer within the past half year, (3) planned or initiated chemotherapy or chemoradiotherapy with/without immunotherapy, (4) could complete body composition analysis, handgrip strength, 6-M step speed, and questionnaires, (5) had not received nutritional support or professional guidance on dietary intake before admission, (6) intended to participate in this study voluntarily. The patients who had previously experienced chemotherapy, were with comorbid neuro-muscular related diseases (such as myasthenia gravis, paralysis, or Parkinson's disease), suffered from severe medical diseases (such as stroke, liver and kidney failure, uncontrolled diabetes mellitus, hyperlipidemia, etc.), or were with the presence of drastic changes in body composition in the last 3 months (such as dehydration, persistent fever, edema, etc.), would be excluded.

Ethics

The study was approved by the accredited Medical Research Ethics Committee in Peking Union Medical College Hospital (no. ZS-3321), and the study procedures were conducted following the Declaration of Helsinki. This study was registered on the Clinical Trial (NCT02873676). All patients provided written informed consent to participate in this study.

Evaluation methods and data collection

Frailty was assessed by the Fried Frailty Phenotype (FFP), consisting of 5 phenotypes: unexplained weight loss, fatigue, decreased grip strength, decreased walking speed, and decreased physical activity, with a score of 0 considered healthy, 1–2 as pre-frailty, and ≥3 as frailty (18). Nutritional risk and malnutrition were assessed by the NRS-2002 and GLIM, respectively (Table 1).

Sarcopenia was diagnosed according to AWGS 2019 criteria. Skeletal muscle mass (SMM) was measured by multi-frequency bioelectric impedance analysis (m-BIA) at fasting state on the second morning after patient's admission. To increase the accuracy of muscle mass assessment, we randomly selected 32 enrolled patients (25.4%) to assess the cross-sectional area (cm^2) at the L3 level of abdominal CT scans within 2 weeks before admission, which have emerged as the golden standard (19). Segmentation of skeletal muscle, including the psoas, erector spinae, quadratus lumborum, transversus abdominis, external and internal obliques, and rectus abdominis muscles, was

TABLE 1 Domains and corresponding screening tools with applied cut-off values (13, 14, 17).

	Tests used	Outcome	Cut-off value
Muscle mass	m-BIA	SMI in cm^2/m^2	Male SMI <7.0 kg/m^2
			Female SMI <5.7 kg/m^2
Muscle strength	Handgrip	Kilogram	Male ≤28 kg, Female ≤18 kg
Muscle function	Walking speed	Meter/second	<1.0 m/s
Frailty	FFP	Score ranged 0–5	Healthy = 0
			Pre-frailty = 1–2
			Frailty ≥3
Nutritional risk	NRS-2002	Score ranged 0–7	No risk = NRS-2002 <3
Malnutrition	GLIM	Status	At least 1 phenotypic criterion and 1 etiologic criterion for diagnosis of malnutrition

m-BIA, multi-frequency bioelectric impedance analysis; FFP, fried frailty phenotype; GLIM, Global Leadership Initiative on Malnutrition Criteria; NRS-2002, Nutritional Risk Screening-2002.

manually performed by Varian Eclipse, using a muscle-specific Hounsfield Unit (HU) range between −29 and +150. Two consecutive images were analyzed to generate the mean of SMA at L3. SMM was calculated according to the following formula (20) and normalized for patient's height to calculate SMI (kg/m^2). Then SMI obtained by the m-BIA and CT was compared. The handgrip strength and 6-M walking speed measurements were used to assess the patients' muscle strength and function.

Total body muscle mass (kg) = 0.3 × skeletal muscle at L3 (cm^2) + 6.06.

Statistics

The baseline characteristics of patients were described. Continuous variables were described using means ± standard deviations (SD), and medians and quartiles for normally and non-normally distributed data, respectively. Ordinal or nominal variables were expressed as absolute values and percentages. Bivariate Pearson's *r* correlation coefficients were used to evaluate the uniformity of SMM measurement by m-BIA and CT, and to analyze the correlation between SMI, sarcopenia, frailty, NRS-2002, and GLIM. Univariate logistic regression analyses were performed to assess whether frailty, nutritional risk, and malnutrition were related to muscle status, with sarcopenia as the dependent variable and baseline variables as independent variables. Possible multicollinearity was analyzed with variance inflation factor (VIF). Variables that were with statistical significance ($p < 0.10$) in univariate regression with VIF <3 were included in the multivariate logistic regression analysis in a backward manner. The strength of association between the variables and sarcopenia was expressed as odds ratios (OR) with corresponding 95% confidence intervals (CI). In the multivariate analysis, a $p < 0.05$ was considered statistically significant. SPSS version 26.0 (IBM SPSS Statistics) was used for statistical analysis.

Results

Demographic characteristics

The average age of the participants was 64.8 ± 8.7 years (range 34–86 years), and 77.0% were male. Pathological types included adenocarcinoma, squamous carcinoma, and small cell lung cancer, with the majority being stage IV (55.5%). Nutritional risk, malnutrition, frailty, sarcopenia (by AWGS 2019), handgrip strength, walking speed, and calf circumference were performed in all patients and shown in Table 2.

According to the AWGS 2019 criteria, the prevalence of sarcopenia was 25.4% ($n = 32$), 20.7% ($n = 6$) in females and 26.8% ($n = 26$) in males. If the assessment was based on SMI alone, then 48 patients (38.1%) had reduced muscle mass (myopenia) with a mean SMI of 7.2 ± 0.9 kg/m^2 in all, and 7.5 ± 0.7 kg/m^2 and 6.4 ± 0.7 kg/m^2 in males and females, respectively ($p < 0.001$). When assessed according to muscle strength or function decline, 43 patients (34.1%) had suspected probable sarcopenia, with a mean handgrip strength of 28.6 ± 8.1 kg and walking speed of 1.12 m/s. According to the FFP questionnaire, 32.5% ($n = 41$) and 43.8% ($n = 55$) of patients were in frailty and pre-frailty status.

Compared to non-sarcopenia patients, the sarcopenic group has elder age, lower BMI, more co-morbidities (aCCI ≥4), higher cancer stage (stage IV), higher prevalence of nutritional risk (NRS-2002 score ≥3), malnutrition, frailty, and ECOG (≥2) ($p < 0.05$), however, gender, pathological type, and NLR were not significantly different between two groups.

The agreement of muscle mass measurements

Thirty-two patients were randomly selected for consistent evaluation of SMI measured by m-BIA and CT. SMI assessed by CT and calculated as 7.0 ± 1.4 kg/m^2, which is comparable to m-BIA result ($r = 0.791$, $p = 0.011$), indicating the SMI measurement using the m-BIA method were reliable in this study.

Correlation analysis of sarcopenia, nutritional risk, malnutrition, and frailty

After correcting for age, gender, and cancer stage, the correlations between sarcopenia, FFP score, NRS-2002 score, and GLIM classification are shown in Table 3. Sarcopenia and frailty were significantly correlated ($p < 0.001$) since both of which focus on muscle strength and function in their respective assessment criteria. Although muscle mass assessment is lacking in frailty, SMI was shown significantly correlated with frailty

TABLE 2 Characteristics of demography and screening tools in participants with and without sarcopenia.

	Total (*n* =126)	Non-sarcopenia (*n* =94, 74.6%)	Sarcopenia (*n* =32, 25.4%)	*p* value
Gender				0.642*
Female	29 (23.0%)	23 (24.5%)	6 (18.8%)	
Male	97 (77.0%)	71 (75.5%)	26 (81.3%)	
Age (years)	64.8 ± 8.7	63.8 ± 8.8	67.6 ± 7.6	**0.031**
<65	53 (42.1%)	44 (46.8%)	9 (28.1%)	
≥65	73 (57.9%)	50 (53.2%)	23 (71.9%)	
BMI (kg/m^2)	24.0 ± 3.1	24.7 ± 2.8	21.7 ± 2.7	**<0.001**
<18.5	5 (4.0%)	1 (1.1%)	4 (12.5%)	
18.5–23.9	58 (46.0%)	36 (38.3%)	22 (68.8%)	
≥24.0	63 (50.0%)	57 (69.6%)	6 (18.8%)	**0.001**
aCCI	2.8 ± 1.4	2.6 ± 1.3	3.3 ± 1.4	**0.007**
0–1		18 (19.1%)	3 (9.4%)	
2–3		60 (63.8%)	15 (46.9%)	
≥4		16 (17.0%)	14 (43.8%)	
Smoking				0.293*
Never	39 (31.0%)	30 (31.9%)	9 (28.1%)	
Active/quit	87 (69.0%)	64 (68.0%)	23 (71.9%)	
SMI (kg/m^2)	7.2 ± 0.9	7.5 ± 0.7	6.4 ± 0.8	**<0.001**
CC (cm)	34.6 ± 3.2	35.6 ± 2.4	31.7 ± 3.2	**<0.001**
VFA (cm^2)	83.0 (60–108)	84.0 (66–112)	62.0 (50–106)	**0.024**#
Handgrip (kg)	28.6 ± 8.1	30.0 ± 7.7	24.4 ± 7.9	**0.001**
Normal	82 (65.1%)	71 (75.5%)	11 (34.4%)	
Decreased	44 (34.9%)	23 (24.5%)	21 (65.6%)	**<0.001**
Walking speed (m/s)	1.2 ± 0.6	1.3 ± 0.3	1.0 ± 0.2	**0.004**
≥1.0	75 (59.5%)	65 (69.2%)	10 (31.3%)	<0.001
<1.0	51 (40.5%)	29 (30.9%)	22 (68.8%)	
Cancer histology				0.349*
Adenocarcinoma	47 (37.3%)	37 (39.4%)	10 (31.3%)	
Squamous-cell carcinoma	41 (32.5%)	30 (31.9%)	11 (34.4%)	
SCLC	38 (30.2%)	27 (28.7%)	11 (34.4%)	
Cancer stage				**0.016***
III	56 (44.4%)	48 (51.1%)	8 (25.0%)	
IV	70 (55.5%)	46 (48.9%)	24 (75.0%)	
NRS-2002	2.1 ± 1.2	1.9 ± 1.1	2.6 ± 1.4	**0.014**
<3	90 (71.4%)	69 (73.4%)	18 (56.3%)	
≥3	36 (28.6%)	25 (26.6%)	14 (43.8%)	
GLIM				**0.004**
Healthy	94 (74.6%)	73 (77.7%)	21 (65.6%)	
Malnutrition	32 (25.4%)	22 (23.4%)	10 (31.3%)	
Mild	26 (20.6%)	17 (18.1%)	9 (28.1%)	
Severe	6 (4.8%)	1 (1.1%)	5 (15.6%)	
NLR	4.3 ± 3.17	3.29 (2.47–4.55)	3.91 (2.64–5.15)	0.220#
FFP	4.6 ± 2.5	4.2 ± 2.2	5.8 ± 2.9	**<0.001**
Healthy	30 (23.8%)	30 (31.9%)	0	
Pre-frailty	55 (43.7%)	40 (42.6%)	15 (46.9%)	
Frailty	41 (32.5%)	24 (25.5%)	17 (53.1%)	
ECOG	1 (0–2)	0 (0–1)	1 (1–2)	**<0.001***
<2	86 (68.3%)	86 (91.5%)	20 (62.5%)	
≥2	20 (15.9%)	8 (8.5%)	12 (37.5%)	

aCCI, age-adjusted Charlson Comorbidity Index; BMI, body mass index; CC, calf circumference; ECOG, Eastern Cooperative Oncology Group; FFP, fried frailty phenotype; GLIM, Global Leadership Initiative on Malnutrition Criteria; NLR, neutrophil-lymphocyte ratio; NRS-2002, nutrition risk screening-2002; SCLC, small cell lung cancer; SMI, skeletal muscle index; VFA, visceral fat area. The non-normal distributed data are presented with median (interquartile range).*$\chi 2$ test.

#Mann–Whitney *U*-test. Bold values denote statistical significance at the $p<0.05$ level.

TABLE 3 Correlation among different evaluation criteria.

	NRS-2002		GLIM		Frailty	
	r	*p*	*r*	*p*	*r*	*p*
Sarcopenia	0.157	0.082	0.446	<0.001	0.335	<0.001
NRS-2002	–		0.525	<0.001	0.357	<0.001
GLIM	–		-		0.453	<0.001

GLIM, Global Leadership Initiative on Malnutrition Criteria.

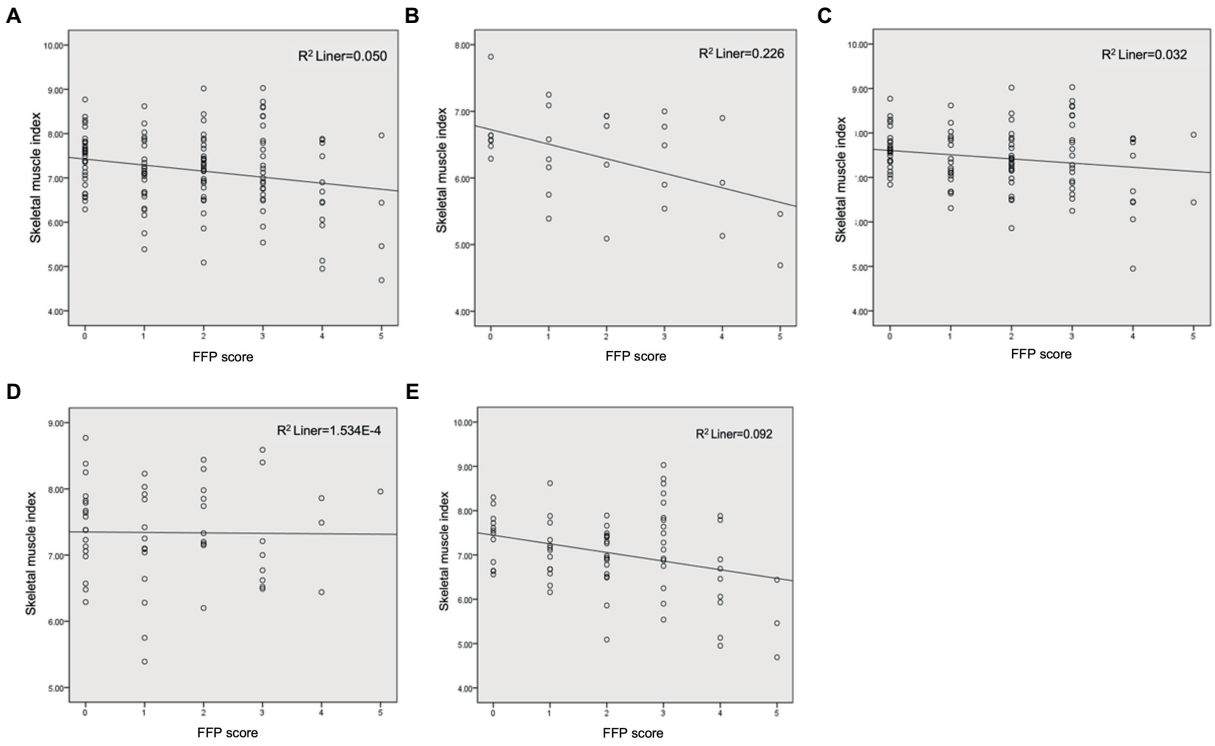

FIGURE 1
Correlation analysis of SMI and FFP. **(A)** Total subjects; **(B)** Female; **(C)** Male; **(D)** <65 years; **(E)** ≥65 years.

scores in this study ($r = -0.204$, $p = 0.027$). Although calf circumference, another measure of muscle mass, showed a negative correlation with frailty, was not statistically significant ($r = -0.063$, $p = 0.602$), because calf circumference may be influenced by body size and cannot accurately reflect SMM. Frailty is also strongly correlated with nutritional risk and malnutrition as they both include an evaluation of recent weight loss. Although the nutrition risk screening lacked an evaluation of muscle status, the correlation between SMI and NRS-2002 scores was significant ($r = -0.230$, $p = 0.013$), while handgrip strength and walking speed were not correlated with the NRS-2002 screen ($r = -0.176$, $p = 0.057$; $r = -0.113$, $p = 0.212$), which could explain the absent of significant correlation between nutritional risk and sarcopenia. Although GLIM did not focus on muscle strength and function, it was significantly correlated with sarcopenia ($r = 0.436$, $p < 0.001$), handgrip strength, and walking speed ($r = -0.239$, $p = 0.008$; $r = -0.197$, $p = 0.030$).

The scatterplots for SMI and FFP revealed a negative correlation for the whole population (Figure 1A). After correcting for age, gender, and cancer stage, the correlation analysis demonstrated both SMI and comprehensively assessed sarcopenia were significantly correlated with FFP scores in all populations ($r = 0.335$, $p < 0.001$; $r = -0.215$, $p = 0.017$). In addition, NRS-2002 and aCCI also showed a positive correlation with FFP ($r = 0.357$, $p < 0.001$; $r = 0.348$, $p < 0.001$). However. BMI, visceral fat, calf circumference, and NLR ($r = -0.078$, $p = 0.406$; $r = 0.109$, $p = 0.245$; $r = -0.045$, $p = 0.629$; $r = 0.129$, $p = 0.157$) were not associated with FFP.

Gender was stratified to clarify the effect of gender on the relationship between SMI and frailty scores. Although scatterplots still showed a negative correlation between SMI and FFP (Figures 1B,C), the correlation analysis, adjusted for age and cancer stage, suggested SMI was not significantly correlated with FFP in females and males ($r = -0.213$, $p = 0.287$; $r = -0.098$, $p = 0.348$), however FFP in men ($n = 97$) showed significant association with sarcopenia ($r = -0.555$, $p < 0.001$). Therefore, it suggested we cannot focus on muscle mass alone, but need to evaluate muscle strength and function to better predict frailty. In women, the intensity of the analysis may have been limited by the sample size.

Data were stratified for the age group to clarify the effect of age on the relationship between SMI and frailty, and scatterplots are illustrated in Figures 1D,E. In the <65-year-old population ($n = 53$), after correction for age, sex, and cancer stage, FFP was associated with

TABLE 4 Univariate and multivariate logistic regression analysis.

Variables		Univariate logistic regression		Multivariate logistic regression	
		OR (95% CI)	*p* value	OR (95% CI)	*p* value
Gender	Female	1			
	Male	1.404 (0.514, 3.833)	0.508		
Age	<65	1			
	≥65	2.249 (0.942, 5.371)	0.068		
BMI (kg/m^2)	18.5–23.9	1	**0.024**[#]	1	**0.017**[#]
	<18.5	6.545 (0.687, 15.213)	0.102	6.095 (1.799, 13.649)	0.244
	≥24.0	0.172 (0.064, 0.466)	**0.001**	0.088 (0.021, 0.372)	**0.042**
aCCI	0–1	1			
	2–3	1.500 (0.390, 5.768)	0.555	1.039 (0.175, 6.155)	0.966
	≥4	2.291 (1.128, 4.654)	**0.022**	3.128 (0.449, 21.778)	0.249
Smoking	Never	1			
	Active/quit	1.198 (0.495, 2.900)	0.689		
Cancer histology	Adenocarcinoma	1			
	Squamous-cell carcinoma	1.553 (0.618, 3.901)	0.349		
	SCLC	1.234 (0.738, 2.063)	0.423		
Cancer stage	III	1			
	IV	3.000 (1.224, 7.353)	**0.016**		
NRS-2002	<3	1			
	≥3	2.147 (0.931, 4.947)	0.073		
GLIM	Healthy	1			
	Malnutrition	3.981 (1.531, 10.358)	**0.005**		
FFP		1.956 (1.402, 2.730)	**<0.001**	1.553 (1.030, 2.343)	**0.036**
ECOG	<2	1		1	
	≥2	6.450 (2.330, 17.858)	**<0.001**	7.286 (1.779, 29.838)	**0.006**
NLR		1.058 (0.938, 1.188)	0.366		

aCCI, age-adjusted Charlson Comorbidity Index; BMI, body mass index; CC, calf circumference; ECOG, Eastern Cooperative Oncology Group; FFP, fried frailty phenotype; GLIM, Global Leadership Initiative on Malnutrition Criteria; NLR, neutrophil-lymphocyte ratio; NRS-2002, nutrition risk screening-2002; SCLC, small cell lung cancer; SMI, skeletal muscle index; VFA, visceral fat area. The non-normal distributed data are presented with median (interquartile range). [#]Overall *p* value of variable. Bold values denote statistical significance at the $p<0.05$ level.

sarcopenia ($r = 0.325$, $p = 0.024$), but not with SMI ($r = 0.048$, $p = 0.748$). In the ≥65-year-old population ($n = 73$), FFP was associated with both sarcopenia and SMI ($r = 0.296$, $p = 0.017$; $r = -0.297$, $p = 0.016$). This indicates that frailty is less related to baseline SMI in people <65 years old, and the close association of frailty and sarcopenia is more likely to be contributed by muscle strength and function, emphasizing the importance of the evaluation of both. However, in the ≥65 years old group, frailty was associated with both muscle mass and quality. Remarkably, in the <65 and ≥65 years old group, frailty was associated with aCCI ($r = 0.380$, $p = 0.008$; $r = 0.351$, $p = 0.004$), NRS-2002 ($r = 0.341$, $p = 0.018$; $r = 0.398$, $p = 0.001$), and malnutrition ($r = 0.405$, $p = 0.004$; $r = 0.463$, $p < 0.001$), indicating the comorbidities and nutritional status should be paid attention to.

Univariate and multivariate logistic regression

Table 4 summarizes the univariate logistic regression analysis with sarcopenia as the dependent variable. More co-morbidities (aCCI ≥4), later cancer stage (stage IV), malnutrition, higher FFP score, and ECOG were risk factors for sarcopenia, whereas BMI ≥24.0 kg/m^2 was a protective factor for sarcopenia ($p < 0.001$). Patients with sarcopenia tend to be male, older (≥65 years), smoking, with lower BMI (<18.5 kg/m^2), with nutritional risk, and with higher NLR compared to patients without sarcopenia, but the association was not statistically significant.

The covariance test showed no covariance among the variables (VIF <2), so age, BMI, aCCI, NRS-2002 score, FFP, ECOG, cancer stage, and GLIM classification were put into the multivariate analysis (see Table 4). The results showed BMI (OR 0.551, 95%CI 0.338–0.897, $p = 0.017$), FFP score (OR 1.553, 95%CI 1.030–2.343, $p = 0.036$), and ECOG (OR 7.286, 95%CI 1.779–29.838, $p = 0.004$) were the influencing factors of sarcopenia.

We aimed to analyze both males and females, but the sample size of female was too small ($n < 50$). In males, univariate regression analysis with sarcopenia as a dependent variable was performed and found BMI (OR 0.678, 95%CI 0.546–0.842, $p < 0.001$), cancer stage (OR 3.627, 95%CI 1.302–10.103, $p = 0.014$), GLIM (OR 4.213, 95%CI 2.562–12.416, $p = 0.001$), and FFP (OR 1.876, 95%CI 1.284–2.740, $p = 0.001$) were significant variables. Multivariate regression analysis in a backward manner revealed FFP (OR 1.536, 95%CI 1.062–2.452,

TABLE 5 Univariate and multivariate logistic regression analysis for male.

Variables		Univariate logistic regression		Multivariate logistic regression	
		OR (95% CI)	*p* value	OR (95% CI)	*p* value
BMI (kg/m^2)		0.678 (0.546, 0.842)	**<0.001**	0.625 (0.479, 0.815)	**0.001**
Cancer stage	III	1			
	IV	3.627 (1.302, 10.103)	**0.014**		
GLIM	Healthy	1	0.001		
	Slight malnutrition	3.750 (1.300, 10.817)	**0.014**		
	Severe malnutrition	5.000 (4.603, 10.216)	**0.001**		
FFP		1.876 (1.284, 2.740)	**0.001**	1.536 (1.062, 2,452)	**0.042**

BMI, body mass index; FFP, fried frailty phenotype; GLIM, Global Leadership Initiative on Malnutrition Criteria. Bold values denote statistical significance at the $p<0.05$ level.

TABLE 6 Univariate and multivariate logistic regression analysis for subjects <65 years old.

Variables		Univariate logistic regression		Multivariate logistic regression	
		OR (95% CI)	*p* value	OR (95% CI)	*p* value
BMI (kg/m^2)		0.521 (0.332, 0.818)	**0.005**	0.428 (0.228, 0.802)	**0.008**
NRS-2002	<3	1			
	≥3	4.229 (0.904, 19.786)	0.067		
GLIM	Healthy	1	0.137		
	Slight malnutrition	5.700 (1.030, 34.950)	**0.046**		
	Severe malnutrition		**0.032**		
FFP		2.172 (1.198, 3.941)	**0.011**	2.919 (1.299, 6.558)	**0.009**

BMI, body mass index; FFP, fried frailty phenotype; GLIM, Global Leadership Initiative on Malnutrition Criteria; NRS-2002, nutrition risk screening-2002. Bold values denote statistical significance at the $p<0.05$ level.

TABLE 7 Univariate and multivariate logistic regression analysis for subjects ≥65.

Variables		Univariate logistic regression		Multivariate logistic regression	
		OR (95% CI)	*p* value	OR (95% CI)	*p* value
BMI (kg/m^2)		0.708 (0.571, 0.877)	**0.002**	0.719 (0.574, 0.900)	**0.004**
Cancer stage	III	1			
	IV	2.614 (0.885, 7.729)	0.082		
GLIM	Healthy	1	0.003		
	Slight malnutrition	3.094 (0.938, 10.201)	**0.046**		
	Severe malnutrition	44.000 (4.621, 78.927)	**0.001**		
FFP		1.769 (1.173, 2.668)	**0.007**	1.701 (1.095, 2.643)	**0.018**

BMI, body mass index; FFP, fried frailty phenotype; GLIM, Global Leadership Initiative on Malnutrition Criteria; NRS-2002, nutrition risk screening-2002. Bold values denote statistical significance at the $p<0.05$ level.

$p = 0.042$) and BMI (OR 0.625 95%CI 0.479–0.815, $p = 0.001$) as independent variables associated with sarcopenia in males (Table 5).

Univariate and multivariate logistic regression analysis was performed stratified by age. For both the <65 and ≥65 years old populations, BMI, GLIM, and FFP were significant variables for each subgroup ($p < 0.05$), while in a multivariate regression analysis in a backward manner, BMI and FFP were independent variables for sarcopenia (Tables 6, 7).

Discussion

This manuscript described the prevalence and investigated the relationship of frailty, sarcopenia, nutritional risk, and malnutrition in 126 hospitalized patients with primary lung cancer. This is the first study that proposes that comprehensively assessed sarcopenia is independently associated with frailty based on FFP in lung cancer inpatients. The results suggest that comprehensive sarcopenia assessment, including muscle mass and quality, is more correlated to frailty, and comprehensive evaluation for sarcopenia may represent frailty status.

Studies over the past have shown that sarcopenia is prevalent in lung cancer patients and is associated with chemotherapy toxicity, tolerance, and short survival (21–24), emphasizing the importance of identifying the sarcopenic group. According to the AWGS 2019 criteria, the incidence of sarcopenia in our study was 25.4%, which was lower than 43–52% in some previous studies (25–27). Firstly, the patients of our studies were newly diagnosed and initially treated

patients, so the shorter duration of illness and the lack of exposure to chemotherapy might be the reason. Secondly, most of the previous studies only reported CT-defined sarcopenia, instead of comprehensive sarcopenia. In this study, the incidence of decreased muscle mass based on SMI was 38.1%, which is also called muscle disorder or myopenia, however, some of this population may have an inherent insufficient of muscle mass, but not represent a strength or functional reduction.

Since patients need regular chest and abdomen CT scan to evaluate the treatment effect, most previous studies on sarcopenia in lung cancer retrospectively reported the SMA and attenuation at the L3 level of CT to describe muscle mass and quality. However, there is a lack of consensus on the standardized thresholds of CT-based SMI used for diagnosing sarcopenia. Nevertheless, in clinical practice, finding recent CT images and then asking imaging physicians to measure SMA and SMD using specialized software is more cumbersome. M-BIA is widely used in body composition assessment in clinics, and there is a strong correlation between its measurements and those assessed by CT (28, 29). Moreover, m-BIA detection is non-invasive, radiation-free, and quick. Therefore, the AWGS 2019 diagnostic criteria includes m-BIA as a method to assess SMM. M-BIA does not measure intermuscular fat or provide the insight into muscle quality, and is susceptible to the patient's hydration status, but the patients in this study were in a fasting, moderately hydrated condition to reduce error. In addition, the actual measurement of handgrip strength and walking speed compensates for the assessment of muscle quality, which has a greater ability than muscle mass to predict poor outcome in sarcopenic patients (12), and can be integrated into the clinical pathway for inpatients, allowing better selection of patients to receive intensive therapy. For this reason, we evaluated sarcopenia strictly according to the AWGS 2019 criteria and demonstrated sarcopenia was closely related to FFP scores, and that FFP, BMI, and ECOG were influencing factors in sarcopenia.

Sarcopenia and frailty are partially overlapping but fundamentally different conditions. Frailty is defined as a state of the significant impact caused by stress; therefore its assessment focuses on psychology, cognitive function, family support, and the subjective feelings of the patients (30). Sarcopenia is a state of a progressive and generalized loss of skeletal muscle mass, strength, and function, and therefore focuses more on physical aspects (31). The FFP score (18) conceptualizes and quantifies weight loss and impaired mobility as potential factors of frailty (32), and sarcopenia uses handgrip strength and walking speed as measures, which may explain the accordance of the two conditions (33, 34). Our findings suggested a strong correlation between sarcopenia and frailty, but this result may be influenced by the evaluation criteria chosen. As demonstrated in a previous study of HNC patients (35), low SMI was associated with G8 scores but not with GFI, because GFI focuses more on the social and cognitive aspects of frailty. Although muscle mass is not addressed in the diagnostic criteria for FFP, we found a significant correlation between SMI and FFP. As known, muscle mass reduction is associated with a decrease in somatic activity function (34). As a result, our results confirm that frailty and sarcopenia are two intersecting and overlapping states in patients with lung cancer (36).

In performing subgroup analyses by gender and age, we found FFP and SMI were closely correlated only in the ≥65-year-old group, while in the <65-year-old and the male subgroup, FFP was only correlated with sarcopenia, but not SMI. Moreover, it has been shown that muscle quality, such as SMD on CT imaging, is more relevant to frailty in older patients than skeletal muscle quantification (33). As a result, muscle strength and function assessment cannot be ignored. The <65-year-old and male groups are with relatively good muscle mass at baseline, and their frailty is more likely to be affected by the disease. In the population aged ≥65 years, FFP was more frequently associated with SMI, sarcopenia, and malnutrition, indicating the importance of maintaining muscle mass and nutrition status in this population.

The prevalence of low BMI was only 4.0%, but the prevalence of inadequate muscle mass was as high as 38.1%, supporting earlier findings that muscle mass is not necessarily related to BMI (20, 37), because cancer patients may be accompanied by an increase of adipose tissue, so weight cannot be used alone to assess nutritional status. However, BMI is still an important influencing factor on sarcopenia since the regression analysis in this study found a larger BMI was a protective factor for sarcopenia, therefore it is important to positively improve the low body weight in oncology patients. Although GLIM was significantly correlated with both sarcopenia and frailty, it was not an influencing factor for sarcopenia in the regression analysis, probably because both NRS-2002 scores and GLIM focused less on muscle quality. This study showed that there was a significant correlation between sarcopenia and frailty, nutritional risk, and malnutrition in lung cancer patients, but only frailty, low BMI, and ECOG were risk factors for sarcopenia in the multivariable regression analysis, and such results did not differ between different gender age groups, indicating the results were not gender or age dependent.

Sarcopenia, malnutrition, and frailty can occur concurrently in cancer patients since they all include the assessment of nutrition status and muscle loss and are related to poor clinical outcomes. Cancer cachexia, proposed by an expert panel in 2012, was defined as a multifactorial syndrome manifested by an ongoing muscle loss (with or without adiposity loss), could not be fully reversed by conventional nutritional support, and contributes to functional impairment (38). The diagnostic criteria of cancer cachexia include malnutrition, loss of muscle mass, and abnormal biochemical markers related to inflammation and metabolic alternations. Since loss of muscle is the common characteristics shared by sarcopenia and cachexia, sometimes they are overlapping. However, cachexia also underlies involuntary weight loss, reduced food intake, and systematic inflammation, while sarcopenia emphasizes the objective manifestation and comprehensively qualitative and quantitative muscle assessment, instead of recent nutritional status alternations. Although sarcopenia was initially regarded as age-related, it has been recently found to be secondary to disease and related to adverse clinical outcomes and physical dysfunction. Moreover, in our study, age was not an influencing factor for cancer-related sarcopenia in lung cancer. Ideally, further studies could follow up the patients to collect changes in muscle mass, treatment-related toxicity, and survival to investigate the clinical outcome most correlated screening scales and influencing factors.

With the understanding of the effects of nutritional risk and muscle on clinical outcomes in lung cancer patients, nutritional intervention should be conducted. In patients with advanced cancer, maintaining skeletal muscle mass and physical function is often challenging and complicated due the anorexia and weakness. Usually, nutrition intervention, including sufficient calory intake, increased protein intake beyond 1.2 g/kg/day, and physical activities are

recommended (39, 40), and some antioxidants, amino acid supplementation, and vitamin D may also help in muscle maintenance. However more studies are required to determine the optimal multimodal interventions and their impact on clinical outcomes in cancer patients.

Our study has several strengths. First, this study was of patients with pre-treatment lung cancer, who were not affected by prior treatment. Second, the prospective study collected comprehensive information on patients, including diet and weight changes, and comorbidities, which were more accurate than in the retrospective study. Third, considering the possible error of BIA on body composition assessment, 25% of the patients were selected randomly to compare the SMI results assessed by BIA and CT. In addition, according to the AWGS 2019 diagnostic criteria, BIA has a clear cut-off value for SMI and is homozygous for Asians. Fourth, we used rigorous criteria for sarcopenia diagnosis and comprehensively assessed muscle mass and quality.

The present study also has some limitations. First, as a cross-sectional study, it is unclear which screening modality or which of this screening questionnaire correlates best with clinical outcomes. Second, although patients were required to conduct BIA in a fasting, well-hydrated state, BIA is still vulnerable to individual differences and would be less inaccurate with BMI beyond the range of 16–34 kg/m^2. Last, although the study included more than 100 patients, the sample size and unicentric characteristic still limited the generalization of the results, so our findings should also be tested in larger sample size and multicenter cohort studies.

Conclusion

This study found a significant relationship between sarcopenia, frailty, nutritional risk, and malnutrition in 126 lung cancer hospitalized patients. Since sarcopenia has the potential to be a cost-effective, non-invasive biomarker for patients with frailty and malnutrition, screening for sarcopenia is useful to screen target patients for further nutrition support. Moreover, comprehensively assessment of muscle mass and quality is more correlated with frailty and should be conducted in clinical practice.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by Medical Research Ethics Committee in Peking Union Medical College Hospital. The patients/participants provided their written informed consent to participate in this study.

Author contributions

FW, H-pW, and KY designed this study and revised the paper. FW and H-nZ conducted the research, analyzed the data, and wrote the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

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.

Publisher's note

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Do dietary supplements prevent loss of muscle mass and strength during muscle disuse? A systematic review and meta-analysis of randomized controlled trials

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Objective: We performed a systematic review and meta-analysis of existing randomized controlled trials (RCTs) to assess whether dietary supplements can prevent loss of muscle mass and strength during muscle disuse.

Methods: We searched the following databases: PubMed, Embase, Cochrane, Scopus, Web of Science, and CINAHL for RCTs assessing the effect of dietary supplements on disuse muscular atrophy without language and time restrictions. Muscle strength and leg lean mass were used as the primary outcome indicators. Muscle cross-sectional area (CSA), muscle fiber type distribution, peak aerobic capacity and muscle volume were used as secondary outcome indicators. The risk of bias was assessed using the Cochrane Collaboration's Risk of Bias tool. Heterogeneity was tested using the I^2 statistic index. Mean and standard deviation of outcome indicators were extracted from the intervention and control groups to calculate effect sizes and 95% confidence intervals, with the significance level set at $P < 0.05$.

Results: Twenty RCTs were included with a total of 339 subjects. The results showed that dietary supplements had no effect on muscle strength, CSA, muscle fiber type distribution, peak aerobic capacity or muscle volume. But dietary supplements have a protective effect on the lean mass of the legs.

Conclusion: Dietary supplements can improve lean leg mass, but did not show a tendency to have an effect on muscle strength, CSA, muscle fiber type distribution, peak aerobic capacity or muscle volume during muscle disuse.

Systematic review registration: https://www.crd.york.ac.uk/PROSPERO/#recordDetails, identifier: CRD42022370230.

KEYWORDS

disuse muscular atrophy, dietary supplements, muscle strength, leg lean mass, meta-analysis

1. Introduction

Patients recovering from an illness or injury usually require a period of bed rest or limb immobilization. However even a short period of inactivity can result in a significant loss of skeletal muscle mass and strength (1, 2), which might lead to a longer recovery period and a higher risk of disease recurrence (3–6). Skeletal muscle is the most abundant tissue in the body, and when it atrophies, it affects the metabolism throughout the body, such as reduced insulin sensitivity (7, 8), decreased basal metabolic rate (9), and increased body fat mass (10). These factors will affect body functions and result in more serious health problems. Therefore, it is necessary to take measures to prevent loss of muscle mass and strength during periods of inactivity.

It is well known that exercise is the best way to maintain and increase muscle mass (11–13). However, in many cases, patients are not allowed to exercise, so other methods are needed to prevent skeletal muscle atrophy. Loss of skeletal muscle mass due to muscle disuse is attributed to a long-term imbalance between muscle protein synthesis and catabolic rates. A decrease in the basal muscle protein synthesis rate has been reported following bed rest (14, 15) and limb immobilization (16, 17). Previous studies found that supplementation with nutrients, such as dietary protein and essential amino acids, can reduce muscle loss and increase muscle growth during immobilization and aging by stimulating muscle protein synthesis (18). The growth of skeletal muscle is traditionally referred to as skeletal muscle hypertrophy, which is manifested as an increase in muscle mass, muscle thickness, muscle area, muscle volume, and muscle fiber cross-sectional area (CSA) (19). However, it remains unclear about the effect of dietary supplements on disuse muscular atrophy. Some randomized controlled trials (RCTs) showed that nutritional supplementation protected against skeletal muscle atrophy during disuse (20, 21), while some RCTs have showed the opposite results (22, 23). Therefore, a meta-analysis of existing RCTs is necessary to provide updated evidence on the effectiveness of dietary supplements in the treatment of disuse muscular atrophy.

2. Methods

This review follows the recommendations of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses and the Cochrane collaboration for systematic reviews (24). It answers the following research question: Do dietary supplements prevent loss of muscle mass and strength during muscle disuse? The study is registered on PROSPERO, and the registration number is CRD42022370230.

2.1. Search strategy

We searched the PubMed, Embase, Cochrane, Scopus, Web of Science, CINAHL databases for RCTs assessing the effect of dietary supplements on disuse muscular atrophy from the year of inception to December 15, 2022 with no language restrictions. The search terms were (disused muscle atrophy OR skeletal muscle disuse atrophy OR muscle disuse OR disuse atrophy OR muscle disuse atrophy OR disuse atrophies OR immobilization OR immobilization-induced atrophy OR bed rest OR bed rests OR rest, bed OR rests, bed) AND (dietary supplements OR diet therapy OR nutrition therapy OR dietary supplement OR supplements, dietary OR dietary supplementations OR supplementations, dietary OR food supplementations OR food supplements OR food supplement OR supplement, food OR supplements, food OR therapy, nutrition OR nutrition OR diet therapies OR therapy, diet) AND (randomized controlled trial OR controlled trial OR clinical trial). The detailed search strategy was shown in Supplementary Table S1. Furthermore, we manually searched the reference lists of eligible studies to identify additional studies.

2.2. Study selection

Two authors (J-MY, YL) independently screened and selected the studies, with disagreements resolved by a third author (HY). The inclusion criteria for the studies were based on the PICOS (patients, intervention, comparison, outcomes, and study design) principle (25), as shown below:

Patients (P): Healthy subjects who were immobilized lower extremity or on prolonged bed rest according to experimental needs.

Intervention (I): Nutritional supplements were available in capsules, tablets, liquid, powder form, or supplements were added to the daily diet. Supplements can include macronutrients, such as proteins, carbohydrates, and fats; and/or micronutrients, such as vitamins.

Comparison (C): The control group was regular diet or placebo.

Outcomes (O): Muscle strength, leg lean mass, CSA, fiber type distribution (%): type I and type II, peak aerobic capacity and muscle volume.

Study design (S): Randomized controlled trial.

Studies were excluded if they met the following criteria:

(1) Individuals were excluded if they had musculoskeletal impairments, tumors, or critically ill, etc. (2) The intervention group provided dietary supplements combined with other physical therapy, or did not include dietary supplements. (3) Outcomes did not include outcome indicators any of interest. (4) The type of studies were not RCTs.

2.3. Data extraction

Relevant data were extracted from the included studies: (1) name of first author; (2) year of publication; (3) study population; (4) number of participants in the intervention and control groups; (5) type of dietary supplements; (6) dietary supplement dosage and duration; (7) age, sex and body mass index of study participants; (8) differences mean and standard deviation (SD) of muscle strength, leg lean mass, CSA, fiber type distribution, peak aerobic capacity, and muscle volume between control and intervention groups. If mean and SD of the differences were not available, we first tried to contact the authors. After no response, we extracted the mean and SD of the pre-intervention and post-intervention values for the control and intervention groups. If the data in the article

was presented as a picture and no response was received after contacting the author for specific values, the WebPlot Digitizer tool (https://apps.automeris.io/wpd/index.zh_CN.html) was used to extract the mean and SD. Data were extracted independently by two authors (YL, HY), and disagreements were resolved by a third author (J-MY).

2.4. Quality assessment of the study

The methodological quality of the included studies was assessed independently by two raters (Y-BZ, YL) using the Physiotherapy Evidence Database (PEDro) scale, with discrepancies resolved via discussion with a third rater (FG). The PEDro scale consists of 11 items encompassing external validity (Item 1), internal validity (Items 2 to 9), and statistical reporting (Items 10 to 11). The items are as follows: (1) eligibility criteria and source, (2) random allocation, (3) concealed allocation, (4) baseline comparability, (5) blinding of participants, (6) blinding of therapists, (7) blinding of assessors, (8) adequate follow-up (>85%), (9) intention-to-treat analysis, (10) between-group statistical comparisons, and (11) reporting of point measures and measures of variability. Items are rated yes or no (1 or 0) according to whether the criterion is clearly satisfied in the study. A total PEDro score is achieved by adding the ratings of Items 2 to 11 for a combined total score between 0 and 10. Studies with scores <4 are considered poor, 4 to 5 are considered fair, 6 to 8 are considered good and 9 to 10 are considered excellent (26).

In addition, we assessed the quality of each outcome according to the Grading of Recommendations Assessment Development and Evaluation (GRADE) criteria (27), assigning ratings of very low, low, moderate, and high, which involved assessing five domains, including risk of bias, directness of evidence, consistency, precision of results, publication bias.

2.5. Risk of bias assessment

The risk of bias for the included RCTs was assessed independently by two authors (J-HZ, YL) using the Cochrane Collaboration Network's Risk of Bias tool (28). The following items were assessed for each study: (1) random sequence generation, (2) allocation concealment, (3) blinding of participants and personnel, (4) blinding of outcome assessment, (5) incomplete outcome data, (6) selective reporting, and (7) other bias. According to the Cochrane Handbook recommendations, each item was classified as low risk, high risk (not fulfilling the criteria) or unclear risk (specific details or descriptions were not reported), and disagreements were discussed with and resolved by a third author (FG).

2.6. Data synthesis and statistical analysis

Statistical analysis was performed using Review Manager 5.3 statistical software. The mean and SD of differences, sample size were input into the statistical software. If the difference values were not directly available and after contacting the authors without a response, they were calculated using Equation ①, where SD (b), SD (f), and SD (d) represent the SD of the baseline, final, and difference values, respectively, with a correlation coefficient R value estimated at 0.8. If the standard error (SE) was provided in the article, after contacting the author to obtain the SD without getting a response, the SD was calculated according to Equation ②, where n represents the sample size. If the median was provided in the article, the median was considered as the mean value. Heterogeneity was tested using the I^2 statistical indicator, and $I^2 > 50\%$ was considered as high heterogeneity (29). If $I^2 > 50\%$, a random-effects model was used; otherwise, a fixed-effects model was used (29, 30). We reported the effect sizes using the weighted mean differences (WMD) or standardized mean differences (SMD) and 95% confidence interval (95% CI). According to the Cochrane Handbook, the choice of WMD and SMD depends on whether the outcome index evaluation criteria are the same, and WMD is chosen when they are the same, while SMD is chosen when they are not. In addition, subgroup analyses were performed to assess whether the results differed across conditions, and sensitivity analyses were performed to assess the robustness of the results.

$$SD(d) = \sqrt{SD(b)^2 + SD(f)^2 - (2 \times R \times SD(b) \times SD(f))} \quad (1)$$

$$SD = SE\sqrt{n} \quad (2)$$

3. Results

3.1. Search results

The flow chart of study selection was shown in Figure 1. After systematic search from the six databases and other sources, 901 studies were identified. Of which 590 remained after removing duplicates literature. Then, after screening the titles and abstracts of the articles, 533 irrelevant articles were excluded, and 57 were left. Finally, a total of 20 studies (20, 21, 23, 31–47)were included for meta-analysis, and 37 studies were excluded after reading the full article for the following reasons: (1) non-healthy subjects ($n = 6$), (2) no outcome of interest ($n = 24$), (3) conference article ($n = 2$), (4) upper extremity immobilization ($n = 4$), (5) data can't be extracted ($n = 1$). The list of literatures exclusion and reasons for exclusion are shown in Supplementary Table S2.

3.2. Characteristics of the included studies

The characteristics of the 20 included RCTs were shown in Table 1. A total of 339 subjects were enrolled, of which about 22% were women, the number of subjects per study ranged from 10 to 30, and their ages ranged from 20 to 70 years. Of the included 20 RCTs, the dietary supplement was leucine in four studies (20, 32, 35, 36), essential amino acids/carbohydrates in the five (41, 43–46), protein in seven (21, 23, 33, 34, 38, 40, 47),

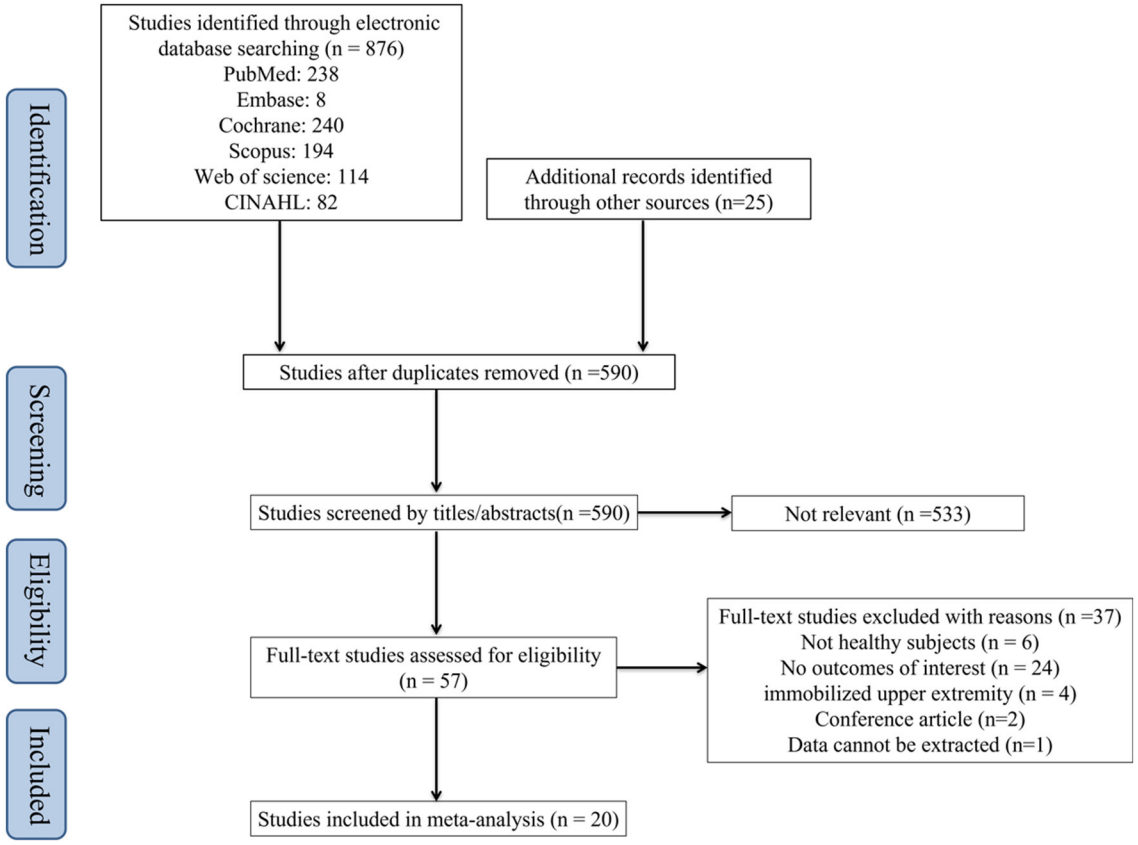

FIGURE 1
Flow chart of study selection.

creatine in two (31, 37), omega-3 fatty acids in one (39), and β-hydroxy-β-methylbutyrate in one (42). Details of the composition, dose and duration of dietary supplementation performed daily in each study were shown in Table 1. Outcome indicators for only two of the twenty RCTs were expressed as mean and SD (39, 45). The outcome indicators of the other eighteen RCTs were expressed as mean and SE (20, 21, 23, 31–38, 40–44, 46, 47). There are a total of seven studies for the outcome indicator of muscle strength gave the mean and SD of differences (20, 21, 35, 36, 40–42), the eight RCTs did not give, and we calculated SD of differences by the Equation (1) (31–34, 37–39, 46). For the outcome indicator of leg lean mass, there are six RCTs gave the mean and SD of differences (20, 36, 41, 42, 44, 46), and three RCTs did not give (39, 43, 47), we calculate the SD of difference according to Equation (1). For the outcome indicator of CSA, two RCTs had mean and SD of differences (40, 45), and five RCTs were calculated by Equation (1) to obtain the SD of difference (31, 32, 34, 37, 39). For the outcome indicator of muscle fiber distribution, the SD of the difference of all four RCTs was calculated (31–34). For the outcome indicator of peak aerobic capacity, three RCTs gave the mean and SD of differences (20, 21, 36), the SD of difference was calculated for the two RCTs (33, 38). For muscle volume, two RCTs gave the mean and SD of differences (39, 45), the SD of difference was calculated for the two RCTs (23, 38). The data for the six RCTs were presented as pictures in the article, and we extracted the mean and SD by using the WebPlot Digitizer tool (33, 34, 38, 40, 42, 45).

3.3. Measurement of outcome indicators

In total, fifteen RCTs measured muscle strength as an outcome indicator. Muscle strength was measured in two ways. Ten RCTs measured knee extensor muscles isometric torque by isokinetic dynamometry (20, 21, 33, 35–40, 42), while the other five RCTs measured knee extension strength by performing one repetition of the maximum strength test on the leg extension machine (31, 32, 34, 41, 46). For the measurement of the lean mass of the legs were all used dual-energy x-ray absorptiometry (20, 36, 39, 41–44, 46, 47). Seven studies reported CSA as an outcome indicator, three of which used CT for detection (31, 32, 34), three studies used MRI for detection (37, 39, 45), and one was detected using Stratec XCT 3000 pQCT with software version 6.20C (40). Outcome indicators of fiber type distribution were taken from the vastus lateralis muscle and then muscle biopsy was performed (31–34). Peak muscle aerobic capacity for the four RCTs was measured by a graded exercise test on a cycle ergometer and metabolic cart (20, 21, 33, 36). Another

TABLE 1 Characteristics of the studies included in the review.

Study	Disuse muscular atrophy	Intervention group			Control group			Outcomes	PEDro Score
		Type	Dose, duration	Population (age, sex, BMI)	Type	Dose, duration	Population (age, sex, BMI)		
Arentson-Lantz et al. (20)	7 days of bed rest	Leucine	14.6 ± 0.8 g leucine/day	Age (y):68 ± 1 Sex (M/F):7/3 BMI (kg/m^2):28.0 ± 1.0	Non-EAA alanine	Isoenergetic control alanine: 13.2 ± 0.5 g/day).	Age (y):68 ± 2 Sex (M/F):7/3 BMI (kg/m^2):25.2 ± 0.7	Knee extensor muscles isometric torque: Nm VO2 peak: L/min Leg lean mass: g	8/10
Arentson-Lantz et al. (21)	7 days of bed rest	Protein	0.90 ± 0.01 g protein/kg/day	Age (y):69 ± 1 Sex (M/F):5/5 BMI (kg/m^2):27.4 ± 0.8	Isoenergetic diets	-	Age (y):68 ± 2 Sex (M/F):7/3 BMI (kg/m^2):25.2 ± 0.7	Knee extensor muscles isometric torque: Nm VO_2 max: L/min	8/10
Backx et al. (32)	7 days immobilization by means of a full leg cast	Leucine	2.5 g leucine, three times daily for 7 days	Age (y):21 ± 1 Sex (M/F):15/0 BMI (kg/m^2):22.7 ± 0.6	7.5 g maltodextrin and 7.5 g dextrose monohydrate	three times daily for 7 days	Age (y):23 ± 1 Sex (M/F):15/0 BMI (kg/m^2):23.5 ± 0.8	Knee Extension Strength (1RM): kg CSA: mm^2 Fiber type distribution (%): type I and type II	9/10
Backx et al. (31)	7 days immobilization by means of a full-leg cast	Creatine	5 g/day creatine for 7 days	Age (y):23 ± 1 Sex (M/F):15/0 BMI (kg/m^2):23.0 ± 0.5	Maltodextrin 7.5 g and dextrose monohydrate 7.5 g	three times daily for 7 days	Age (y):23 ± 1 Sex (M/F):15/0 BMI (kg/m^2):23.5 ± 0.9	Knee Extension Strength (1RM): kg CSA: mm^2 Fiber type distribution (%): type I and type II	7/10
Bosutti et al. (33)	21 days 6° head down-tilt bed rest	Whey protein plus Potassium bicarbonate	0.6 g whey protein/kg body mass/day and 90 mmol KHCO3/day	Age (y):31.6 ± 6.2 Sex (M/F):5/0 BMI (kg/m^2):23.4 ± 1.6	A basic protein diet	1.2 g protein/kg body mass /day	Age (y):31.6 ± 6.2 Sex (M/F):5/0 BMI (kg/m^2):23.4 ± 1.6	Knee extensor muscles isometric torque: Nm Fiber type distribution (%): type I and type II VO_2 max: L/min	7/10
Deutz et al. (42)	10 days of bed rest	β-hydroxy-β-methylbutyrate	3 g/day	Age (y):64 ± 1.4 Sex (M/F):3/8 BMI (kg/m^2):28.63 ± 4.03	inactive placebo powder	-	Age (y):67.1 ± 1.7 Sex (M/F):1/7 BMI (kg/m^2):26.5 ± 1.2	Knee extensor muscles isometric torque: Nm Leg lean mass: kg	9/10
Dirks et al. (34)	5 days immobilization by means of a full-leg cast	Protein	20.7 g of protein, 9.3 g of carbohydrate, and 3.0 g of fat twice daily	Age (y):68 ± 1 Sex (M/F):11/0 BMI (kg/m^2):26.4 ± 0.8	-	-	Age (y):70 ± 1 Sex (M/F):12/0 BMI (kg/m^2):27.3 ± 0.6	Knee extension strength (1RM): kg CSA: mm^2 Fiber type distribution (%): type I and type II	6/10
Edwards et al. (35)	7 days immobilization by means of a full-leg cast	Leucine	15 g leucine/d for 7 days	Age (y):22 ± 1 Sex (M/F):8/0 BMI (kg/m^2):23.8 ± 0.83	Nonessential EAA	15 g placebo/d (non-EAA) for 7 days	Age (y):23 ± 1 Sex (M/F):8/0 BMI (kg/m^2):22.3 ± 0.9	Knee extensor muscles isometric strength: N	9/10
English et al. (36)	14 days of bed rest	Leucine	0.06 g/kg /meal, three times daily	Age (y):51 ± 1 Sex (M/F):6/4 BMI (kg/m^2):24.6 ± 0.9	Alanine control	-	Age (y):52 ± 1 Sex (M/F):6/3 BMI (kg/m^2):24.7 ± 1.7	Knee extensor muscles isometric torque: Nm VO_2 peak: L/min Leg lean mass: kg	9/10

(Continued)

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TABLE 1 (Continued)

Study	Disuse muscular atrophy	Intervention group			Control group			Outcomes	PEDro Score
		Type	Dose, duration	Population (age, sex, BMI)	Type	Dose, duration	Population (age, sex, BMI)		
Ferrando et al. (43)	10 days of bed rest	EAA	15 g of EAA/day	Age (y):71 ± 6 Sex (M/F):1/9 BMI (kg/m^2): -	A placebo	-	Age (y):68 ± 5 Sex (M/F):6/6 BMI: (kg/m^2):	Leg lean mass: kg	5/10
Fitts et al. (44)	28 days of bed rest	EAA and carbohydrate	16.5 g EAA and 30 g sucrose thrice daily	Age (y):36 ± 4 Sex (M/F):5 BMI: (kg/m^2): -	Only the diet soft drink	-	Age (y):38 ± 3 Sex (M/F):5 BMI (kg/m^2):	Leg lean mass: g	5/10
Hespel et al. (37)	14 days immobilization by means of a full-leg cast	Creatine	5 g of creatine monohydrate four times per day	$n = 11$	Only maltodextrin containing citrate (40 mg /g)		$n = 11$	CSA:cm^2 knee extensor muscles isometric torque: Nm	9/10
Holloway (45)	7 days of immobilization by means of a full-leg cast	a proprietary EAA	23.7 g EAA, thrice daily	$n = 10$	an excipient- and energy-matched placebo (maltodextrin) liquid drink	-	$n = 10$	CSA: mm^2 Muscle Volume: mm^3	8/10
Kilroe et al. (38)	3 days immobilization by means of a full-leg cast	Protein	1.6 g/kg body mass /day	Age (y):22 ± 1 Sex (M/F):11/0 BMI (kg/m^2):23 ± 1	Protein	0.15 g/kg body mass /day	Age (y):20 ± 1 Sex (M/F):11/0 BMI (kg/m^2):23 ± 1	Knee extensor muscles isometric torque: Nm VO_2peak: mL/kg/min muscle volume: cm^3	7/10
McGlory et al. (39)	14 days immobilization by means of a full-leg cast	Omega-3 fatty acid	5 g/d of n-3 fatty acid	Age (y):22 ± 3 Sex (M/F):0/11 BMI (kg/m^2):23.1 ± 2.1	Isoenergetic quantity of sunflower oil		Age (y):22 ± 3 Sex (M/F):0/9 BMI (kg/m^2):23.9 ± 2.5	Knee extensor muscles isometric torque: Nm CSA: mm^2 Leg lean mass: g muscle volume: cm^3	10/10
Mitchell et al. (40)	14 days immobilization by means of a full-leg cast	protein	Once daily: 20g milk protein concentrate	Age (y):51.5 ± 3.8 Sex (M/F):15/0 BMI (kg/m^2):27.5 ± 3.2	Once daily: isoenegetic placebo	-	Age (y):48.5 ± 2.4 Sex (M/F):15/0 BMI (kg/m^2):28.3 ± 3.2	Knee extensor muscles isometric torque: Nm CSA: mm^2	10/10
Paddon-Jones et al. (41)	28 days of bed rest	EAA/carbohydrate supplement	16.5 g EAA and 30 g carbohydrate per day	Age (y):36 ± 10 Sex (M/F):7/0	Placebo	-	Age (y):38 ± 8 Sex (M/F):6/0	Knee Extension Strength (1RM): kg Leg lean mass: g	6/10
Paddon-Jones et al. (46)	28 days of bed rest	EAA and carbohydrate	16.5 g EAA and 30 g sucrose	Age (y):36 ± 10 Sex (M/F):7/0 BMI (kg/m^2):	The diet soft drink	-	Age (y):38 ± 8 Sex (M/F):6/0 BMI (kg/m^2): -	Leg lean mass: g Knee Extension Strength (1RM): kg	5/10
Rudwill et al. (47)	21 days of bed rest	High-protein intake, (33% whey protein)	1.8 g/kg/day	Age (y):31 ± 2.1 Sex (M/F):9/0 BMI (kg/m^2):23.9 ± 0.5	Isocaloric control diet	-	Age (y):31 ± 2.1 Sex (M/F):9/0 BMI (kg/m^2):23.8 ± 0.5	Leg lean mass: kg	5/10
Trappe et al. (23)	60 days of bed rest	protein and free leucine	1.45 g/kg body weight /day + 3.6 g/day of free leucine	Age (y):29 ± 1 Sex (M/F):0/8 BMI (kg/m^2): -	Protein	1.0 g/kg body weight/day	Age (y):34 ± 1 Sex (M/F):0/8 BMI (kg/m^2):	Muscle volume: cm^3	5/10

BMI, Body Mass Index; M/F, Male/Female;1RM, one-repetition maximum; CSA, muscle cross-sectional area; VO_2 peak, peak ae ; EAA, Essential amino acid.

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RCT measure of peak aerobic capacity was the single-leg ramp exercise test (38). The muscle volume was all measured by MRI (23, 38, 39, 45).

3.4. Quality of the studies

The methodological quality of the included studies was shown in Table 1 and Supplementary Table S3. Eight studies (20, 21, 31, 33, 34, 38, 41, 45) were rated as good quality. 7 studies (32, 35–37, 39, 40, 42) were rated as excellent quality, and remaining 5 articles were rated fair quality (23, 43, 44, 46, 47). The results indicated insufficient level of concealed allocation, and the sample sizes included in the study were small. The GRADE evidence for the outcome indicators was rated as low, as detailed in Table 2.

3.5. Risk of bias of the studies

The risk of bias of the 20 included studies was shown in Figure 2. Twelve studies were rated as high risk of bias for selection bias because they only mentioned the randomization but did not specify which method was used for random assignment (20, 31–37, 41, 42, 44, 46). Information on allocation concealment was not available in sixteen studies and was therefore rated as unclear risk of bias (20, 21, 23, 31–37, 41, 43–47). Most of the studies on performance bias and detection bias were described as double-blinded and therefore rated as low risk, while those not described were rated as unclear risk of bias. All studies had follow-up rates above 85%, so attrition bias was rated as low risk of bias. Nine studies' reporting bias were rated as low risk because the study protocols were consistent with the outcome indicators in the studies (20, 21, 23, 31, 32, 41–43, 45). And ten studies were rated as unclear risk of bias when there is no enough information about selective reporting (33–40, 44, 46). Other risks of bias that were not mentioned or could not be judged according to the study and were rated as unclear risks. The results of the sensitivity analysis were shown in Supplementary Figure S3.

3.6. Effect of dietary supplements on muscle strength in disuse muscular atrophy

A total of 15 studies measured muscle strength (20, 31–41). Overall meta-analysis showed that dietary supplements had no protective effect on muscle strength during muscle disuse (SMD: 0.19; 95% CI:−0.04, 0.42; p: 0.11). As the types of dietary supplements in both intervention group and control group were heterogenous among the included studies, we performed subgroup analysis to test the effect of different types of dietary supplements on muscle strength. Firstly, we divided the dietary supplements in the intervention group into protein, amino acid, and other nutrients. Other nutrients include β-hydroxy-β-methylbutyric acid, creatine and Omega-3 fatty acids. The results of subgroup analysis (Figure 3) showed that neither protein (SMD: 0.03; 95% CI: −0.36, 0.41; p: 0.89), amino acids (SMD: 0.24; 95% CI: −0.16, 0.63; p: 0.24) nor other nutrients (SMD: 0.34; 95% CI: −0.10, 0.77; p: 0.13) had a protective effect on muscle strength. In addition, we performed a subgroup analysis based on the use of placebo and non-placebo in the control group. Supplementary Figure S1 showed that the use of different supplement types in the control group had no effect on the results.

3.7. Effect of dietary supplements on leg lean mass in disuse muscular atrophy

A total of 9 studies reported on leg lean mass as an outcome indicator (20, 36, 39, 41–44, 46, 47). Overall meta-analysis showed that dietary supplements had protective effect on leg lean mass during muscle disuse (WMD: 0.20; 95% CI: 0.09, 0.31; p: = 0.0003). As the types of dietary supplements in both intervention group and control group were heterogenous among the included studies, we performed subgroup analysis to test the effect of different types of dietary supplements on leg lean mass. We divided the dietary supplements in the intervention group into protein, amino acid, and other nutrients. But the protein group had only one article and meta-analysis was not possible (47). The results of subgroup analysis (Figure 4) showed that the amino acid group significantly improved the lean mass of the legs (WMD: 0.20; 95% CI: 0.08, 0.31; p: = 0.0007), but the other group did not show an effect (WMD: 0.32; 95% CI: −0.19, 0.82; p: 0.22). In addition, we performed a subgroup analysis based on the use of placebo and non-placebo in the control group. Supplementary Figure S2 showed that the use of different supplement types in the control group had no effect on the results.

3.8. Effect of dietary supplements on secondary outcome indicators in disuse muscular atrophy

3.8.1. CSA and muscle fiber type distribution

The results of the meta-analysis of CSA and muscle fiber type distribution are shown in Figure 5. When skeletal muscle atrophy occurs, the CSA becomes smaller. Dietary supplement group did not exhibit greater CSA compared to the control group (SMD: 0.10; 95% CI: −0.20, 0.41; p: 0.55). When skeletal muscle atrophy occurs, type I muscle fibers are converted to type II muscle fibers (48, 49). And then the distribution of type I and type II muscle fibers was altered. As shown in Figure 5, dietary supplementation had no effect on the distribution of type I (WMD: 1.00; 95% CI: −4.58, 6.58; p: 0.73) and type II (WMD: −1.27; 95% CI: −4.58, 6.58; p: 0.49) muscle fibers.

3.8.2. Peak aerobic capacity

When skeletal muscle atrophy occurs, the peak aerobic capacity of the muscle becomes poor. However, as shown in Figure 5, meta-analysis showed that dietary supplementation did not improve the peak aerobic capacity of the disuse muscular atrophy process (SMD: −0.03; 95% CI: −0.45, 0.38; p: 0.88).

TABLE 2 GRADE evidence profile for outcomes among trials included in the systematic review.

No. of studies (participants)	Design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	Absolute effect	Quality	Importance
Muscle strength									
15 (299)	RCTs	Serious*	No serious inconsistency	No serious indirectness	Serious†	None	SMD 0.19 higher (0.05 lower to 0.42 higher)	Low	Critical
Leg lean mass									
9 (157)	RCTs	Serious*	No serious inconsistency	No serious indirectness	Serious†	None	MD 0.39 higher (0.2 to 0.58 higher)	Moderate	Critical
CSA									
7 (170)	RCTs	Serious*	No serious inconsistency	No serious indirectness	Serious†	None	SMD 0.1 higher (0.2 lower to 0.41 higher)	Low	Important
Type I muscle fiber distribution (%)									
4 (88)	RCTs	Serious*	No serious inconsistency	No serious indirectness	Serious†	None	WMD 1 lower (4.58 lower to 6.58 higher)	Low	Important
Type II muscle fiber distribution (%)									
4 (88)	RCTs	Serious*	No serious inconsistency	No serious indirectness	Serious†	None	WMD 1.27 lower (4.92 lower to 2.37 higher)	Low	Important
Peak aerobic capacity									
5 (91)	RCTs	Serious*	No serious inconsistency	No serious indirectness	Serious†	None	SMD 0.03 lower (0.44 lower to 0.39 higher)	Low	Important
Muscle volume									
4 (78)	RCTs	Serious*	No serious inconsistency	No serious indirectness	Serious†	None	MD 62.85 lower (78.28 to 47.42 lower)	Low	Important

*Inadequate allocation concealment; †The sample size was too small ($n < 400$).

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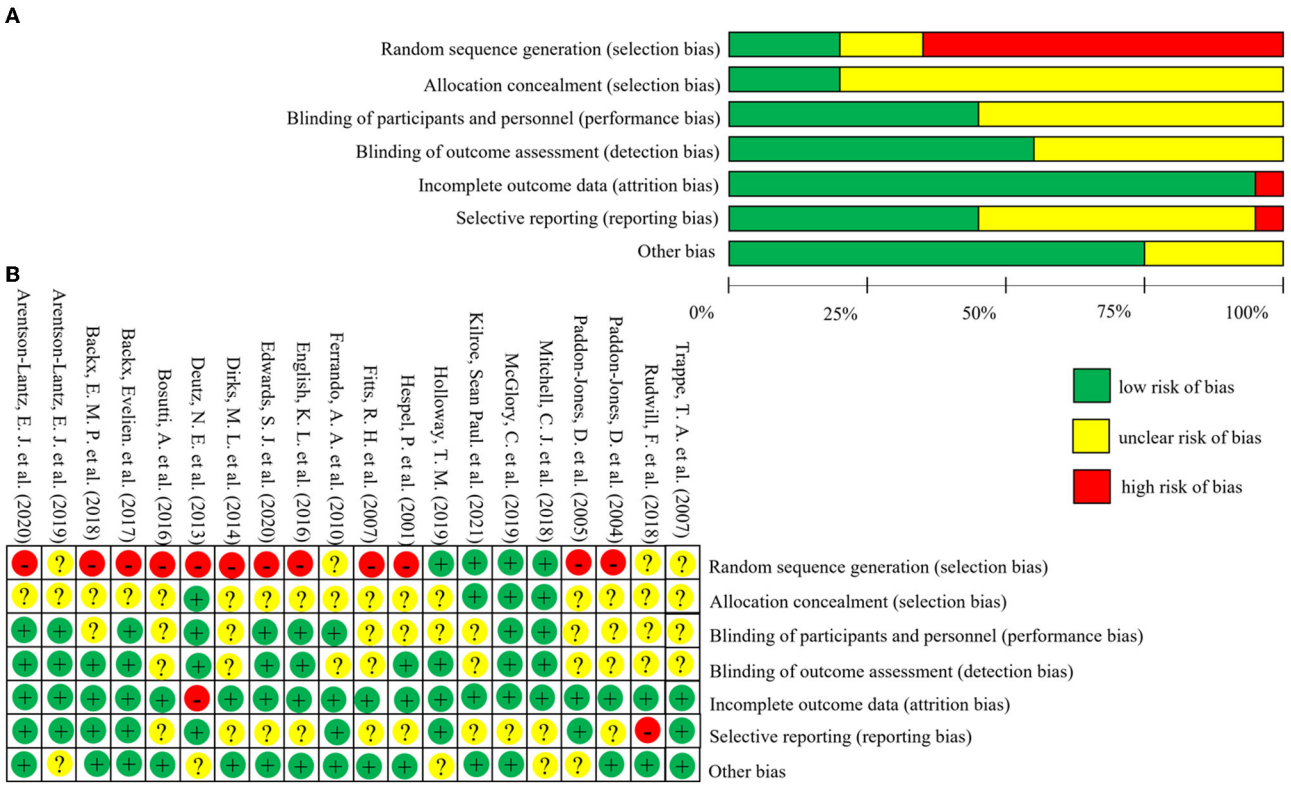

FIGURE 2
Risk of bias graph and summary of included studies. The figure **(A)** (risk of bias graph) shows the overall risk of bias in each domain. The figure **(B)** (risk of bias summary) indicates the risk of bias in each domain for each study.

3.8.3. Muscle volume

As shown in Figure 5, meta-analysis showed that dietary supplements had no effect on muscle volume during muscle disuse (WMD: −33.19; 95% CI: −86.78, 20.40; *p*: 0.22).

4. Discussion

In this systematic review and meta-analysis, we included 20 RCTs with a total of 339 subjects to analyze whether dietary supplements prevent loss of skeletal muscle mass and strength during muscle disuse. The results of the meta-analysis showed that dietary supplements had no effect on muscle strength, but could improve the lean mass of the legs. For the two primary outcome indicators, muscle strength and lean mass, we performed subgroup analysis based on different types of supplements in the control and intervention groups. The results of all subgroup analyses of muscle strength showed that protein, amino acids and other dietary supplements did not improve muscle strength during muscle disuse. Subgroup analyses of leg lean mass showed that the amino acid group significantly improved lean leg mass during muscle disuse, but the other group did not show an effect. However, their total result was that dietary supplements could improve leg lean mass during muscle disuse. This may be related to the relatively small number of studies in the other group. Our results did not support dietary supplementation had effect on secondary outcome indicators including CSA, muscle fiber type distribution, peak aerobic capacity and muscle volume.

Transient muscle disuse after injury or during recovery from disease results in a loss of muscle mass and function, including a progressive decrease in muscle strength, lean muscle mass, muscle volume, aerobic capacity, CSA, atrophy of type I muscle fibers and a shift from type I to type II muscle fibers (50). It is accompanied by many other negative health consequences, such as reduced insulin sensitivity (7, 8), decreased basal metabolic rate (9), and increased body fat mass (10). Therefore, it is important to find a safe and effective measure to alleviate skeletal muscle atrophy during muscle disuse. Our findings suggest that dietary supplementation can play a role in maintaining lean body mass during muscle disuse. Dietary supplements may be considered to maintain lean muscle mass when patients are unable to exercise during muscle disuse. Overall, the risk of bias in our included RCTs was low, and the heterogeneity of the outcome indicators was small. Therefore, our conclusions may provide some guidance for clinical practice.

To our knowledge, this is the first systematic review and meta-analysis of the role of nutritional interventions on disuse muscular atrophy. Our results are consistent with previously published systematic reviews of nutritional interventions for elderly or sarcopenic patients, where nutritional supplementation alone had no effect on muscle strength (51–53). This may be related to the fact that muscle strength is determined by many factors, such as neuromuscular control and muscle mass. However, studies have found that nutritional interventions combined with

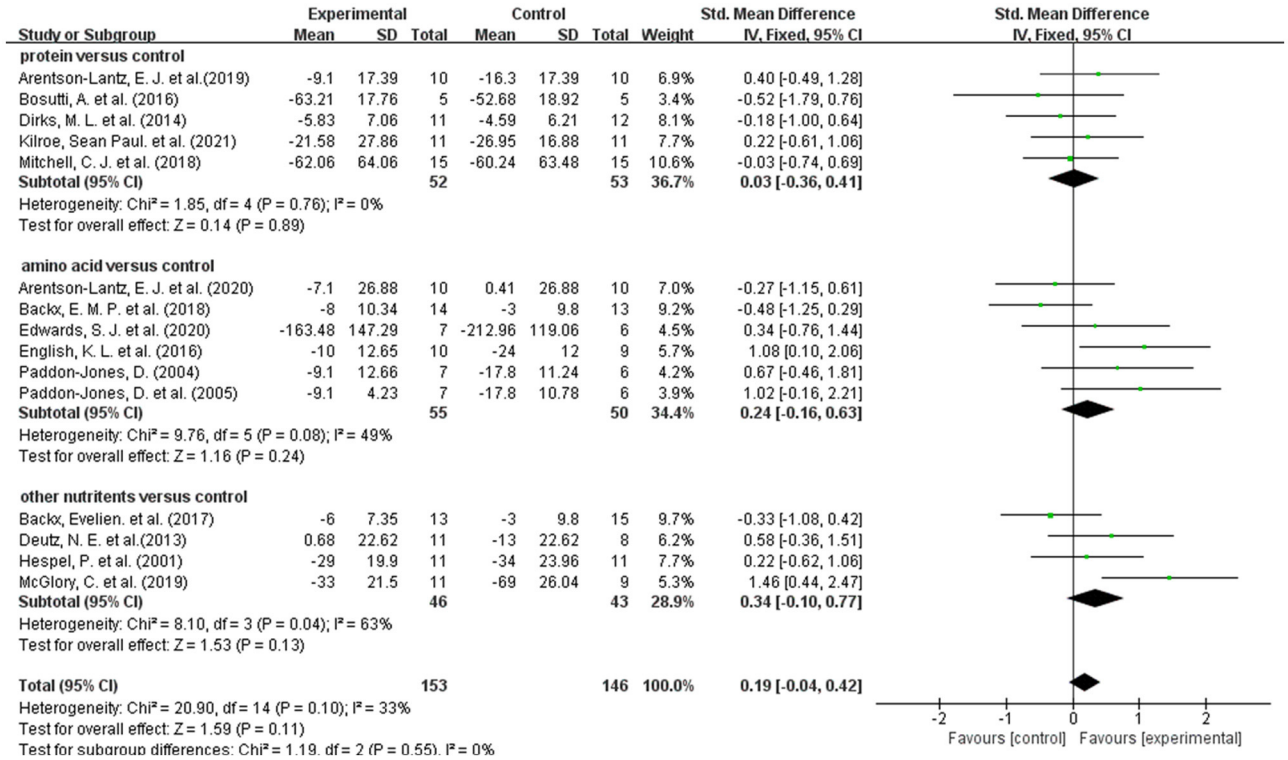

FIGURE 3
Forest plot of subgroup analysis of SMD difference and 95% confidence intervals for the effect of dietary supplements on muscle strength according to the type of experimental group.

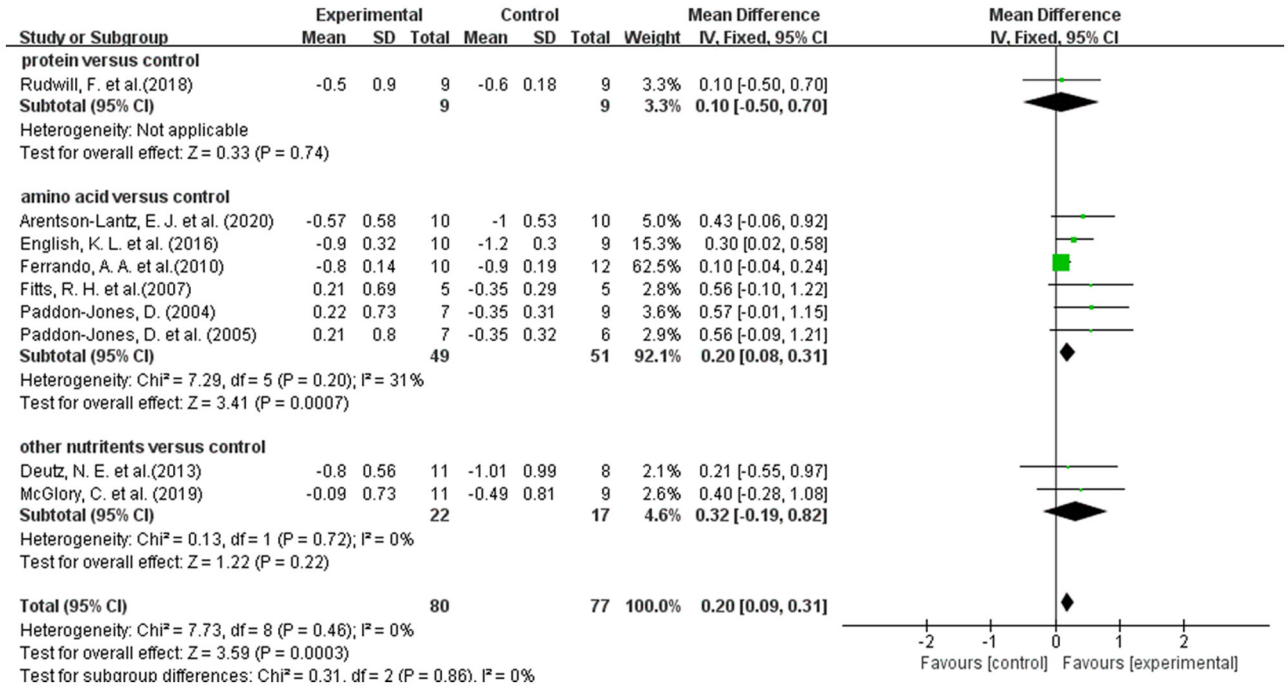

FIGURE 4
Forest plot of subgroup analysis of WMD difference and 95% confidence intervals for the effect of dietary supplements on leg lean mass according to the type of experimental group.

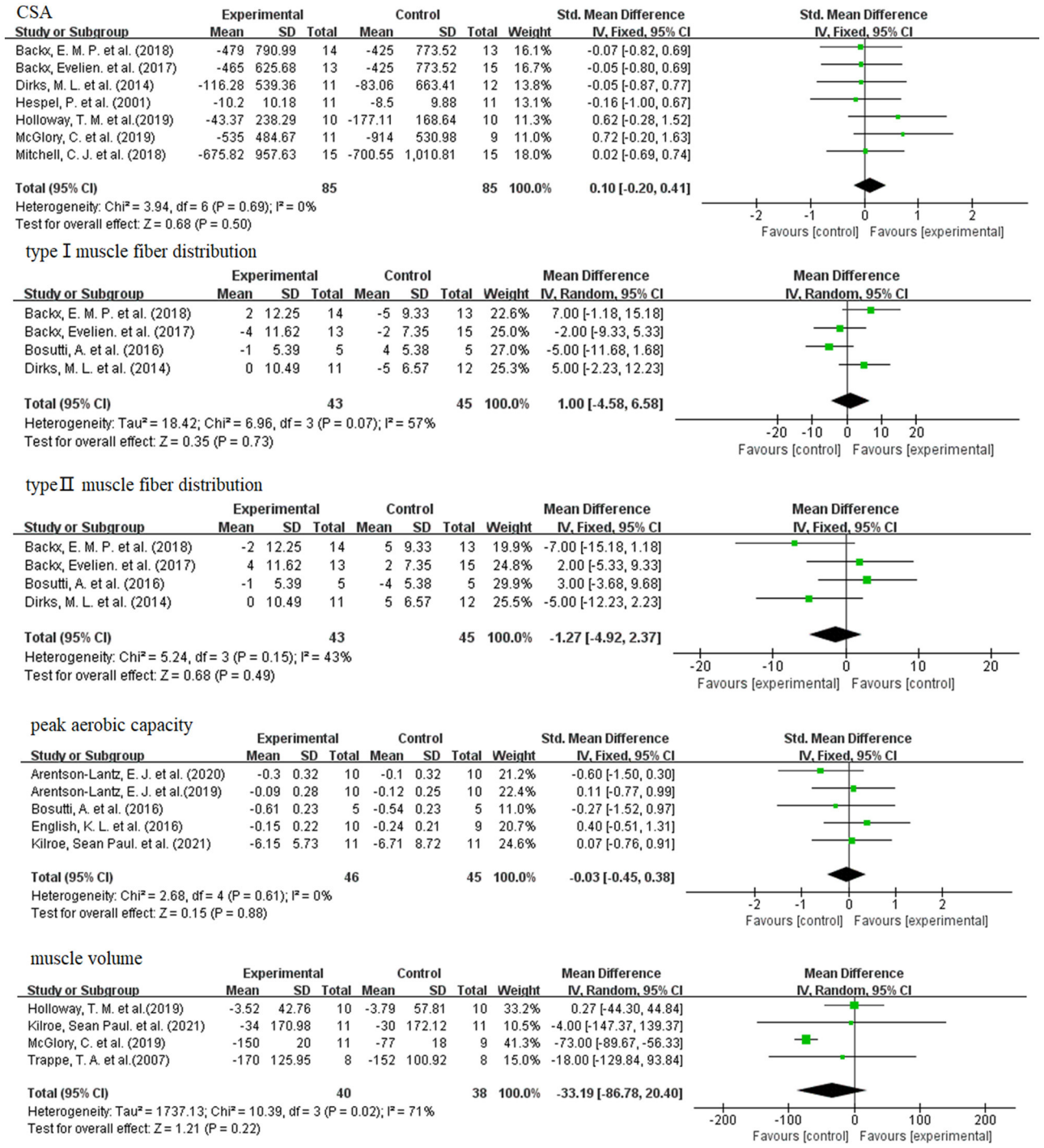

FIGURE 5
Forest plots of SMD, WMD and 95% confidence intervals for the effect of dietary supplements on CSA, type I muscle fiber distribution (%), and type II muscle fiber distribution (%), peak aerobic capacity, and muscle volume.

exercise training can improve muscle strength and physical function (53–56). Therefore, we recommend that nutritional supplementation combined with exercise training interventions should be performed to improve muscle strength after the release of exercise contraindications. For lean mass, the results of our meta-analysis are inconsistent with previously published systematic reviews of nutrition interventions in older adults (52, 57), which may be related to the different populations we included. Our systematic review included healthy subjects, whereas their systematic review included older adults, whose body composition would have changed.

However, this study has some limitations. Firstly, the sample sizes of the included studies in the systematic review were relatively small. Secondly, the number of studies on outcome indicators such as CSA, muscle fiber type distribution, peak aerobic capacity and muscle volume was relatively small. Third, the number of studies

for subgroup analysis was also small. Fourth, some of the RCTs we included do not directly give the SD of the difference, which we calculated by the formula and may differ from the actual value. In addition, some of the outcome indicators are presented in the form of pictures, and the results we obtained by using the WebPlot Digitizer tool to extract the mean and SD may also be different from the actual values. Fifth, the types of dietary supplements in both intervention group and control group were heterogenous among the included studies. Therefore, more RCTs with larger sample sizes are needed in the future to validate the effects of dietary supplements on these outcome indicators.

5. Conclusion

Dietary supplements can improve the lean mass of the legs during muscle disuse. However, our results do not support the role for dietary supplements on muscle strength, CSA, muscle fiber type distribution, peak aerobic capacity or muscle volume.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding authors.

Author contributions

Conception and design and drafted the manuscript: HY, J-MY, and Y-BZ. Collection and analysis of the data: YLu, YLo, and J-HZ. Final approval of the article: FG and M-YW. All authors contributed to the article and approved the submitted version.

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Conflict of interest

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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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2023.1093988/full#supplementary-material

SUPPLEMENTARY FIGURE S1
Forest plot of subgroup analysis of SMD difference and 95% confidence intervals for the effect of dietary supplements on muscle strength according to the type of control group.

SUPPLEMENTARY FIGURE S2
Forest plot of subgroup analysis of SMD difference and 95% confidence intervals for the effect of dietary supplements on leg lean mass according to the type of control group.

SUPPLEMENTARY FIGURE S3
Results of sensitivity analysis on muscle strength, leg lean mass, CSA, type I muscle fiber distribution (%), and type II muscle fiber distribution (%), peak aerobic capacity, and muscle volume.

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Dietary inflammatory potential is associated with sarcopenia in patients with hypertension: national health and nutrition examination study

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Background: Study has shown that sarcopenia increases the risk of poor outcomes in patients with hypertension. Inflammation is one of the important reasons for the occurrence and development of sarcopenia. Regulating systemic inflammation may be a potential intervention for sarcopenia in hypertensive patients. Diet is one of the important measures to improve systemic inflammation. The dietary inflammatory index (DII) is a tool designed to assess the inflammatory potential of the diet, the association between DII and sarcopenia in hypertensive patients is unclear.

Objective: To explore the relationship between the DII and sarcopenia in patients with hypertension.

Method: Data from the National Health and Nutrition Examination Survey (NHANES) 1999–2006 and 2011–2018. A total of 7,829 participants were evaluated. Participants were divided into four groups based on the quartile of the DII: Q1 group (n =1,958), Q2 group (n =1,956), Q3 group (n =1,958) and Q4 group (n =1,957). The relationship between the DII and sarcopenia was assessed by logistic regression analysis based on the NHANES recommended weights.

Result: The DII was significantly associated with sarcopenia in patients with hypertension. After full adjustment, patients with higher DII (OR: 1.22, 95% CI: 1.13–1.32, p <0.001) have a higher risk of sarcopenia. Compared with Q1 group, the group with higher DII levels had a higher risk of sarcopenia (Q2: OR: 1.23, 95%CI: 0.89–1.72, p =0.209; Q3: OR: 1.68, 95%CI: 1.20–2.35, p =0.003; Q4: OR: 2.43, 95%CI: 1.74–3.39, p <0.001).

Conclusion: High DII is associated with an increased risk of sarcopenia in hypertensive patients. The higher the level of DII, the higher the risk of sarcopenia in hypertensive patients.

KEYWORDS

hypertension, sarcopenia, dietary inflammatory potential, NHANES, inflammatory

Introduction

Hypertension is a disease characterized by elevated blood pressure that can cause damage to multiple target organs (1) and is currently thought to be an inflammation-related disease (2). Many studies have found that tumor necrosis factor alpha (TNF-α), C-reactive protein (CRP), chemokine and other inflammatory markers increase abnormally in hypertensive patients (3–5). Interestingly, activation of these inflammatory markers may interfere with cellular protein synthesis via the nuclear factor kappa-B (NF-κB) pathway, contributing to the development of sarcopenia (6). The activation of NOD-like receptor protein 3 (NLRP3) induced by inflammation is also one of the important pathways leading to the decline of muscle fibers (7). Sarcopenia is a degenerative disorder that is estimated to affect 50 million individuals globally is becoming increasingly prevalent (8). Research has demonstrated that people with hypertension are more likely to suffer from sarcopenia (9, 10), and those with both conditions have been found to be at greater risk of cognitive impairment (11), falling incidents (10), and albuminuria (12). Hence, it is necessary to prevent sarcopenia in hypertensive patients.

Given the association between inflammation and hypertension and sarcopenia, controlling inflammation may be a potential intervention to prevent sarcopenia in hypertensive patients. Diet is one of the important measures to control inflammation throughout the body. Energy, saturated fats and trans fats in foods increase levels of markers of inflammation throughout the body (TNF-α, CRP and IL-6) (13, 14). Vitamin E and omega-3 fatty acid intake were associated with decreased levels of markers of inflammation throughout the body (15). However, previous studies have proposed that individual dietary components are difficult to assess overall levels of dietary inflammation in patients due to the diversity of foods (16). In order to assess the overall level of dietary inflammation in patients, previous studies constructed dietary inflammatory index (DII) where high levels of DII represent higher inflammatory dietary potential, low levels of DII represent higher anti-inflammatory dietary inflammatory potential (17). Due to chronic kidney disease (CKD) and Crohn's disease are both associated with inflammation, previous studies have used DII to assess the risk of developing sarcopenia in these patients (18, 19). However, few studies have examined the relationship between DII and sarcopenia in hypertensive patients.

The purpose of our study was to examine whether the risk of sarcopenia differs among hypertensive patients with different DII levels, and to provides some insights into the prevention of sarcopenia in hypertensive patients.

Methods

Study population

The National Health and Nutrition Examination Survey (NHANES) is a comprehensive research project intended to evaluate the health and nutrition status of adults and children in the United States. Sampling approximately 5,000 individuals from various counties across the country every 2 years. Each participant was assigned a different sampling weight. After a complex sampling weighted analysis, these participants were able to represent the entire U.S. population. Due to the lack of skeletal muscle mass data between 2007 and 2010, the study was limited to participants from the years 1999 to 2006 and 2011 to 2018. In NHANES 1999–2006 and 2011–2018, there were 17,874 hypertensive patients over 20 years of age. A total of 9,773 participants lacking skeletal muscle mass and body mass index (BMI) data were deleted. 265 participants lacked dietary data to calculate DII and were excluded. 7 participants lacked dietary weight data and were excluded. After excluding these people, 7,829 people were eventually included in our study (Figure 1).

Definition of hypertension

Hypertension is diagnosed according to the following three items: 1. According to the query posed in the NHANES: "Has a doctor ever told you that you have hypertension?," and "Whether you are taking blood pressure medication," those who answered "yes" were deemed to be hypertensive. 2. the systolic blood pressure (SBP) was measured in the mobile examination center and during home examinations on all eligible individuals using a mercury sphygmomanometer, participants with SBP greater than 140 mmHg or diastolic blood pressure (DBP) higher than 90 mmHg were regarded as hypertensive. In the event that the patient has multiple blood pressure readings, the average is used to make a diagnosis of hypertension. 3. Based on patient self-reported prescriptions, patients are considered hypertensive if they are currently taking calcium channel blockers (CCBS), beta blockers, diuretics, angiotensin-converting enzyme inhibitors, and/or angiotensin II receptor blockers (ACEIs/ ARBs). Participants who met one of these criteria were considered to have high blood pressure. This is consistent with previous research (20).

Primary outcome

The primary endpoint was sarcopenia. Dual-energy X-ray absorptiometry (DXA) whole-body scans were used to appendicular skeletal muscle mass was measured using DXA. Whole body DXA scans were taken with a Hologic QDR-4500A fanbeam densitometer (Hologic, Inc., Bedford, Massachusetts). Hologic software version 8.26:a3* was used to administer all scans. Further details of the DXA examination protocol are documented in the Body Composition Procedures Manual located on the NHANES website: (https://search.cdc.gov/search/index.html?query=DXA&siteLimit=NCHS&dpage=1).

As recommended by the Foundation for National Institutes of Health Osteoarthritis Biomarkers study (FNIH), use the ratio of total appendicular skeletal muscle mass (in kg) to BMI (kg/m^2) to determine if a patient has sarcopenia. The cut-off values for the diagnosis of sarcopenia were not identical (0.789 for men and 0.512 for women) due to physiological differences between men and women (21). This cut-off value was obtained by classification and regression tree (CART) analysis in previous studies (22). Many studies have used this standard to define sarcopenia (23–25).

Calculation of the DII

Dietary inflammation index was designed as an exposure variable. The dietary data in NHANES were obtained by a 24 h dietary recall interview at the mobile examination center (MEC). In our study, carbohydrates, protein, total fat, alcohol, fiber, cholesterol, saturated

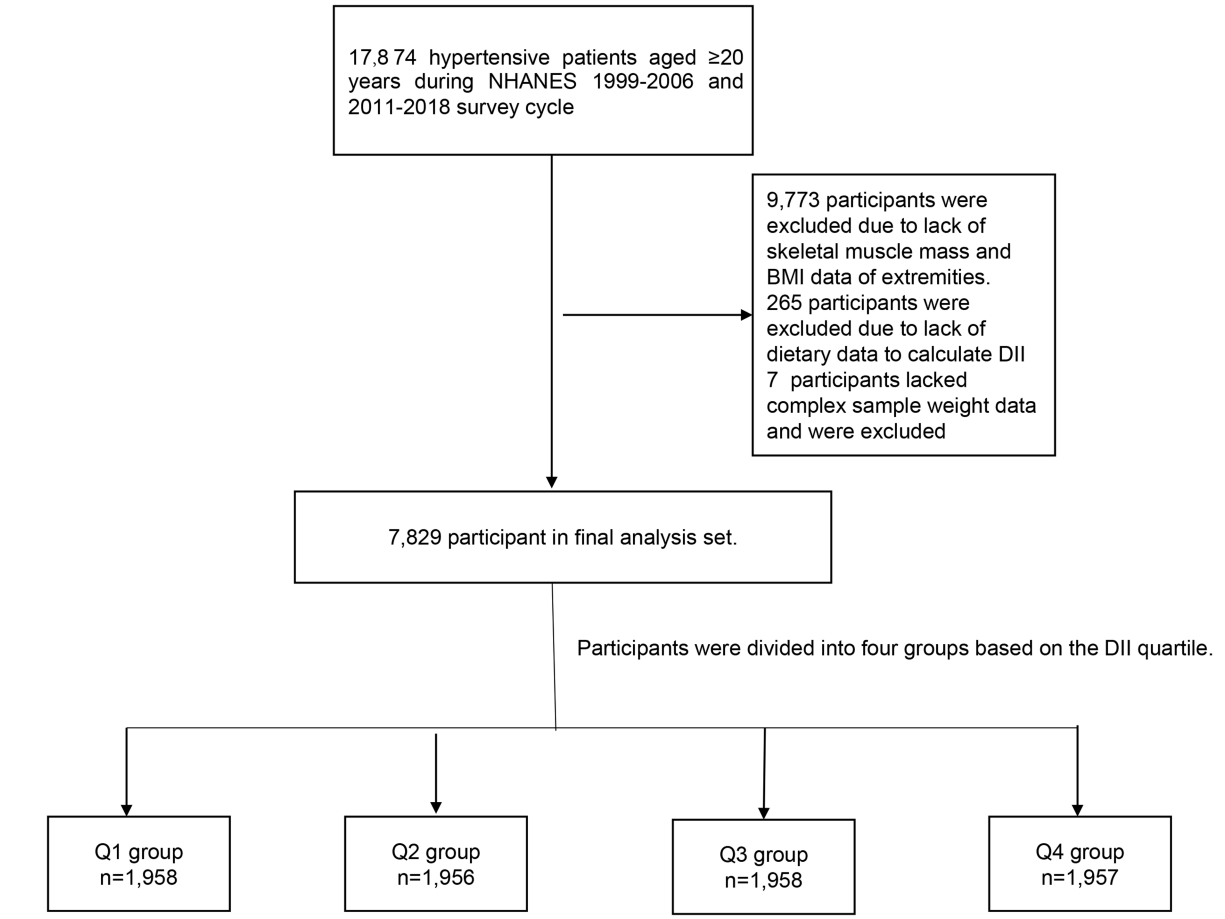

FIGURE 1
Flowchart of the study design.

fatty acids (SFAs), monounsaturated fatty acids (MUFAs), polyunsaturated fatty acids (PUFAs), omega-3 fatty acids, omega-6 fatty acids, niacin, vitamin A, vitamin B1, vitamin B2, vitamin B6, vitamin B12, vitamin C, vitamin D, vitamin E, iron, magnesium, zinc, selenium, folic acid, beta-carotene, caffeine, and energy were used to calculate DII. DII for each nutrient or dietary ingredient = [(daily intake of that nutrient or dietary ingredient -global *per capita* daily intake of that nutrient or dietary ingredient)/that nutrient or dietary ingredient Standard deviation of global *per capita* daily intake] x inflammatory effect index of that nutrient or dietary ingredient, and the sum of DII of each nutrient or dietary ingredient was the total DII score of individual study subjects (26). The anti-inflammatory or proinflammatory parameters of each food can be looked up in the study of Nitin Shivappa et al. (17).

Confounding variable

The selection of confounding variables was determined based on previous studies. Studies have shown that these variables affect the occurrence and development of sarcopenia and need to be adjusted by incorporating regression models (27–30).

Age, sex, race, education level, poverty income ratio (PIR), smoking status and alcohol use were self-reported by participants. BMI was calculated based on the height and weight of the participants. Diagnosis of comorbidities was based on an affirmative response to the question "Has a doctor or other health professional ever told you that you had diabetes mellitus (DM), CKD, cardiovascular disease [CVD (include coronary heart disease, congestive heart failure, heart attack, stroke and angina)]?." Participants were also considered diabetic if they were being treated for diabetes, or had a hemoglobin a1c (HbA1c) of 6.5 percent or more. In addition, participants with estimated glomerular filtration rate (eGFR) <60 mL/min/1.73 m^2 and/or randomized urinary albumin/creatinine ratio (ACR) >30 mg/g were also considered patients with CKD (31). Laboratory measurements, such as triglycerides (TC), total cholesterol (TG) and C-reactive protein (CRP) were collected using automated hematological analysis equipment. Urine albumin was measured by fluorescence immunoassay. Urinary creatinine was measured using Roche/Hitachi modular P chemical analyzer Detailed procedures for obtaining laboratory measurements were provided in a document on the website[1] of the National Center for Health Statistics. In addition, muscle loss caused by statins, sulfonylureas and glycinates was also defined as confounding variables (32–34). Self-reported prescription data was used to determine if the patient was taking these medications, which were defined as other drugs.

1 https://wwwn.cdc.gov/nchs/data/nhanes/2009-2010/manuals/lab.pdf

Method of grouping

The independent variable DII was included as a grouping variable for the purpose of the study, which is consistent with previous studies (35–37). Patients were divided into four groups based on the quartile of DII: Group 1 (DII < 0.35), Group 2 (0.35 ≤ DII <1.82), Group 3 (1.82 ≤ DII <2.90), Group 4 (DII ≥ 2.90).

Statistical analyses

According to the National Health and Nutrition Examination Survey (NHANES) recommended weights, the weights for specific groups were calculated. Continuous variables were expressed as the mean (standard error), and categorical variables were presented as counts (percentages). Baseline characteristics between the different groups were compared using an analysis of variance (ANOVA) for continuous variables, and a $\chi 2$ test for categorical variables.

We conducted logistic regression analyses to assess the association between DII and sarcopenia. All statistical analyses were performed with complex sampling weighted analysis using the weights recommended by NHANES. In order to enhance the robustness of the results, three models were analyzed. Model 1 was the unadjusted model. Model 2 was adjusted for age, gender, and race. Model 3 was fully adjusted for potential confounders, including age, gender, race, smoking status, drinking status, education level, PIR, BMI, TG, TC, the use of antihypertensive drugs, other drugs, DM, CVD, and CKD. To investigate the potential non-linear relationship between DII and sarcopenia, a regression cubic spline (RCS) analysis was also conducted. The adjustment variables for the RCS are consistent with Model 3. In addition, we stratified the analysis by age, sex, and

TABLE 1 Baseline study population characteristics (weighted).

Characteristics	Overall (*N* =7,829)	Q1 group (*N* =1,958)	Q2 group (*N* =1,956)	Q3 group (*N* =1,958)	Q4 group (*N* =1,957)	*p*-value
Age, years	51.4(0.3)	50.9(0.5)	51.6(0.5)	52.4(0.4)	50.9(0.4)	0.017
Female, *n* (%)	3,872(47.9)	699(33.6)	917(46.6)	1,036(53.1)	1,220(61.1)	< 0.001
Race, *n* (%)						< 0.001
Mexican American	1,329(6.6)	355(7.3)	349(7.3)	315(5.3)	310(6.3)	
Non-Hispanic Black	1,961(13.6)	389(10.1)	433(12.3)	544(15.1)	595(17.5)	
Non-Hispanic White	3,478(68.2)	905(70.3)	899(68.7)	869(68.9)	805(64.3)	
Other Hispanic	463(5.1)	124(5.1)	115(5.0)	106(4.8)	118(5.6)	
Other Race	598(6.5)	185(7.2)	160(6.7)	124(5.9)	129(6.2)	
Education, *n* (%)						< 0.001
<12	2,323(19.5)	469(14.3)	549(18.0)	635(22.5)	670(24.0)	
12	1,886(25.9)	421(21.2)	449(24.0)	500(29.0)	516(30.2)	
>12	3,612(54.7)	1,067(64.5)	955(58.1)	820(48.5)	770(45.8)	
DII	1.42(0.04)	−0.92(0.04)	1.11(0.01)	2.37(0.01)	3.57(0.02)	< 0.001
SBP, mmHg	134.6(0.4)	133.8(0.8)	135.1(0.6)	135.6(0.6)	134.2(0.6)	0.249
Smoking, *n* (%)	3,916(51.4)	977(50.7)	955(50.1)	993(52.0)	991(53.1)	0.609
Drinking, *n* (%)	4,879(68.6)	1,339(75.2)	1,273(69.2)	1,194(67.3)	1,073(61.5)	< 0.001
BMI, kg/m^2	30.00(0.12)	29.62(0.19)	29.53(0.20)	30.60(0.21)	30.32(0.23)	< 0.001
TG, mmol/L	1.98(0.03)	2.05(0.08)	1.94(0.05)	1.97(0.05)	1.92(0.04)	0.502
TC, mmol/L	5.31(0.02)	5.27(0.03)	5.29(0.04)	5.34(0.04)	5.35(0.04)	0.313
CRP, mg/dL	0.48(0.02)	0.37(0.02)	0.48(0.04)	0.47(0.03)	0.60(0.03)	< 0.001
PIR	3.00(0.04)	3.35(0.06)	3.10(0.06)	2.86(0.06)	2.61(0.06)	< 0.001
DM, *n* (%)	1,737(17.4)	402(15.1)	426(16.4)	453(17.9)	456(20.8)	0.005
CKD, *n* (%)	1,871(18.7)	393(15.4)	481(18.8)	484(19.2)	513(22.0)	0.003
CVD, *n* (%)	1,218(12.9)	269(10.9)	287(12.7)	312(13.4)	350(15.1)	0.009
Antihypertensive drug, *n* (%)	4,111(49.2)	1,008(46.5)	1,012(49.4)	1,040(50.7)	1,051(50.8)	0.198
Other drugs, *n* (%)	1,643(20.3)	404(19.1)	429(20.6)	410(21.6)	400(19.9)	0.575
Sarcopenia, *n* (%)	1,352(17.3)	265(9.4)	309(10.9)	349(15.3)	429(19.3)	< 0.001

Values are, *n* (%) or mean (SE).
DII, Dietary inflammatory index; SBP, systolic blood pressure; BMI, body mass index; TG, triglyceride; TC, total cholesterol; PIR， poverty income ratio; DM, diabetes mellitus; CKD, Chronic kidney disease. CVD, cardiovascular disease.

antihypertensive drug use, and analyzed whether there was an interaction between DII and these subgroups.

All data analyses were performed by using the Survey package in R software (version 4.2.2; R Foundation for Statistical Computing, Vienna, Austria). A two-sided *p*-value <0.05 indicated significance for all analyses.

Results

Participant characteristics

The baseline clinical characteristics are reported in Table 1. In this study, 7,829 patients with hypertension were enrolled, with an average age of 51.4 (0.3) years, and 3,872 (47.9%) of the participants being female. In total, 1,871 (18.7%) had CVD, 1,737 (17.4%) had DM, 4,111 (49.2%) were routinely taking antihypertensive drugs, and 1,352 (17.3%) had sarcopenia. Participants were divided into four groups based on the DII quartile [Q1 (n = 1,958); Q2 (n = 1,956); Q3 (n = 1,958); Q4 (n = 1,957)]. There was a statistically significant difference in mean age [Q1: 50.9(0.5) vs. Q2: 51.6(0.5) vs. Q3: 52.4(0.4) vs. Q4: 50.9(0.4), p = 0.017] between the four groups. Furthermore, those with higher DII were more likely to be female [Q1: 33.6% vs. Q2: 46.6% vs. Q3: 53.1% vs. Q4: 61.1%, p < 0.001] and had lower education level [education level > 12; Q1: 64.5% vs. Q2: 58.1% vs. Q3: 48.5% vs. Q4: 45.8%, p < 0.001] and PIR [Q1: 3.4 (0.1) vs. Q2: 3.1 (0.1) vs. Q3: 2.9 (0.1) vs. Q4: 2.6 (0.1), p < 0.001] and were less likely to drink [Q1: 75.2% vs. Q2: 69.2% vs. Q3: 67.3% vs. Q4: 61.5%, p < 0.001]. Additionally, those with higher DII had higher CRP [Q1: 0.37(0.02) vs. Q2: 0.48(0.04) vs. Q3: 0.47(0.03) vs. Q4: 0.60(0.03), p < 0.001] and a higher prevalence of DM [Q1: 15.1% vs. Q2: 16.4% vs. Q3: 17.9% vs. Q4: 20.8%, p = 0.005], CKD [Q1: 15.4% vs. Q2: 18.8% vs. Q3: 19.2% vs. Q4: 20.0%, p = 0.003], and CVD [Q1: 10.9% vs. Q2: 12.7% vs. Q3: 13.4% vs. Q4: 15.1%, p = 0.009]. No significant differences were found in smoking (p = 0.609), SBP (p = 0.249), TG (p = 0.502), TC (p = 0.313), use of antihypertensive drugs (p = 0.198) and other drugs (p = 0.575) among the four groups. Further detailed information is shown in Table 1.

The association between DII and sarcopenia

As shown in Figure 2, the group with higher DII levels had a higher prevalence of sarcopenia (Q1 group:9.4% vs. Q2 group:10.9% vs. Q3 Group: 15.3% vs. Q4 group: 19.3). Univariate logistic regression analysis showed that DII (OR: 1.22, 95% CI: 1.15–1.30, p < 0.001) was significantly associated with sarcopenia in patients with hypertension. Compared with Q1 group, the group with higher DII levels had a higher risk of having sarcopenia (Q2: OR: 1.17, 95%CI: 0.97–1.51, p = 0.214; Q3: OR: 1.74, 95%CI: 1.35–2.24, p < 0.001; Q4: OR: 2.29, 95%CI: 1.75–3.01, p < 0.001). After adjusting for age, sex, race, smoking status, drinking status, education, PIR, BMI, TG, TC, antihypertensive drug, other drugs, DM, CVD and CKD, the association between DII (OR: 1.22, 95% CI: 1.13–1.32, p < 0.001) and sarcopenia did not change. Patients with higher DII have a higher risk of having sarcopenia (Q2: OR: 1.23, 95%CI: 0.89–1.72, p = 0.209; Q3: OR: 1.68, 95%CI: 1.20–2.35, p = 0.003; Q4: OR: 2.43, 95%CI: 1.74–3.39, p < 0.001) (Table 2).

Subgroup analysis

After stratifying the participants according to age (p for interaction = 0.999), gender (p for interaction = 0.813) and antihypertensive drug (p for interaction = 0.243), the association between DII and sarcopenia did not change. Compared with Q1 group, the groups with higher DII have higher the risk of developing sarcopenia (Figure 3).

Regression cubic splines

After stratifying the participants according to gender and antihypertensive drug, no potential non-linear relationship was observed between DII and sarcopenia in hypertensive patients. However, there was a non-linear relationship between DII and sarcopenia in hypertensive patients in the subgroup ≥65 years of age (Non-linear p = 0.011) but not in the subgroup <65 years of age (Non-linear p = 0.987) (Figure 4).

Discussion

In this cross-sectional study, our results show that DII is associated with the risk of sarcopenia in hypertensive patients. The higher the DII score, the higher the patient's risk of sarcopenia. There was no significant change in the association between DII and sarcopenia in hypertensive patients after stratified analysis based on age, sex, and antihypertensive drugs.

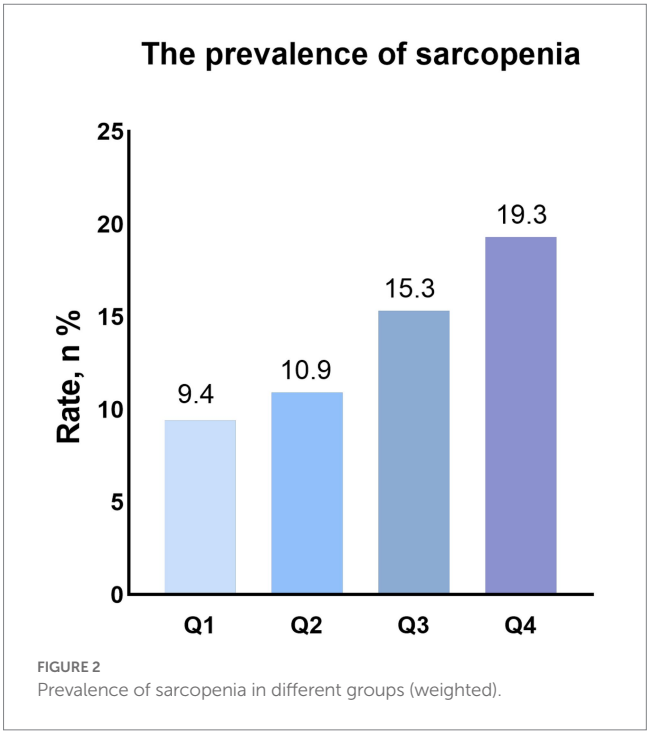

FIGURE 2
Prevalence of sarcopenia in different groups (weighted).

TABLE 2 The association between DII and sarcopenia in patients with hypertension (weighted).

Variable		Model 1		Model 2		Model 3	
		OR (95%CI)	*p*-value	OR (95%CI)	*p*-value	OR (95%CI)	*p*-value
Continuous variables							
DII		1.22(1.15–1.30)	<0.001	1.29(1.21–1.37)	<0.001	1.22(1.13,1.32)	<0.001
Categorical variable	Event/All population						
Q1 group	265/1958	Ref		Ref		Ref	
Q 2 group	309/1956	1.17(0.91–1.51)	0.214	1.21(0.93–1.58)	0.161	1.23(0.89–1.72)	0.209
Q 3 group	349/1958	1.74(1.35–2.24)	<0.001	1.95(1.49–2.55)	<0.001	1.68(1.20–2.35)	0.003
Q 4 group	429/1957	2.29(1.75–3.01)	<0.001	2.88(2.15–3.85)	<0.001	2.43(1.74–3.39)	<0.001

Model 1: Not adjusted.
Model 2: Adjusted by age, gender, race/ethnicity.
Model 3: Adjusted by age, gender, race/ethnicity, smoking status, drinking status, education, PIR, BMI, TG, TC, antihypertensive drug, other drugs, DM, CVD, CKD.

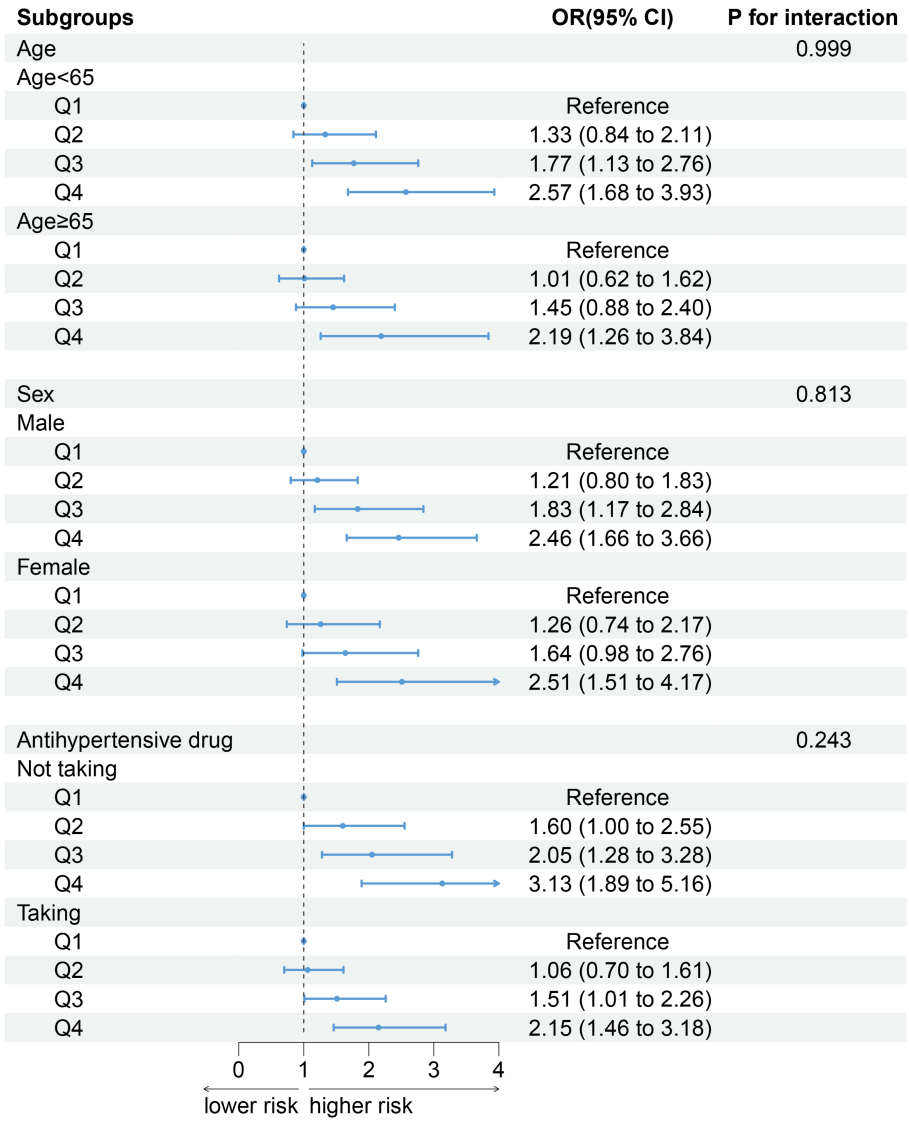

FIGURE 3
Association between DII and sarcopenia by selected subgroups (weighted).

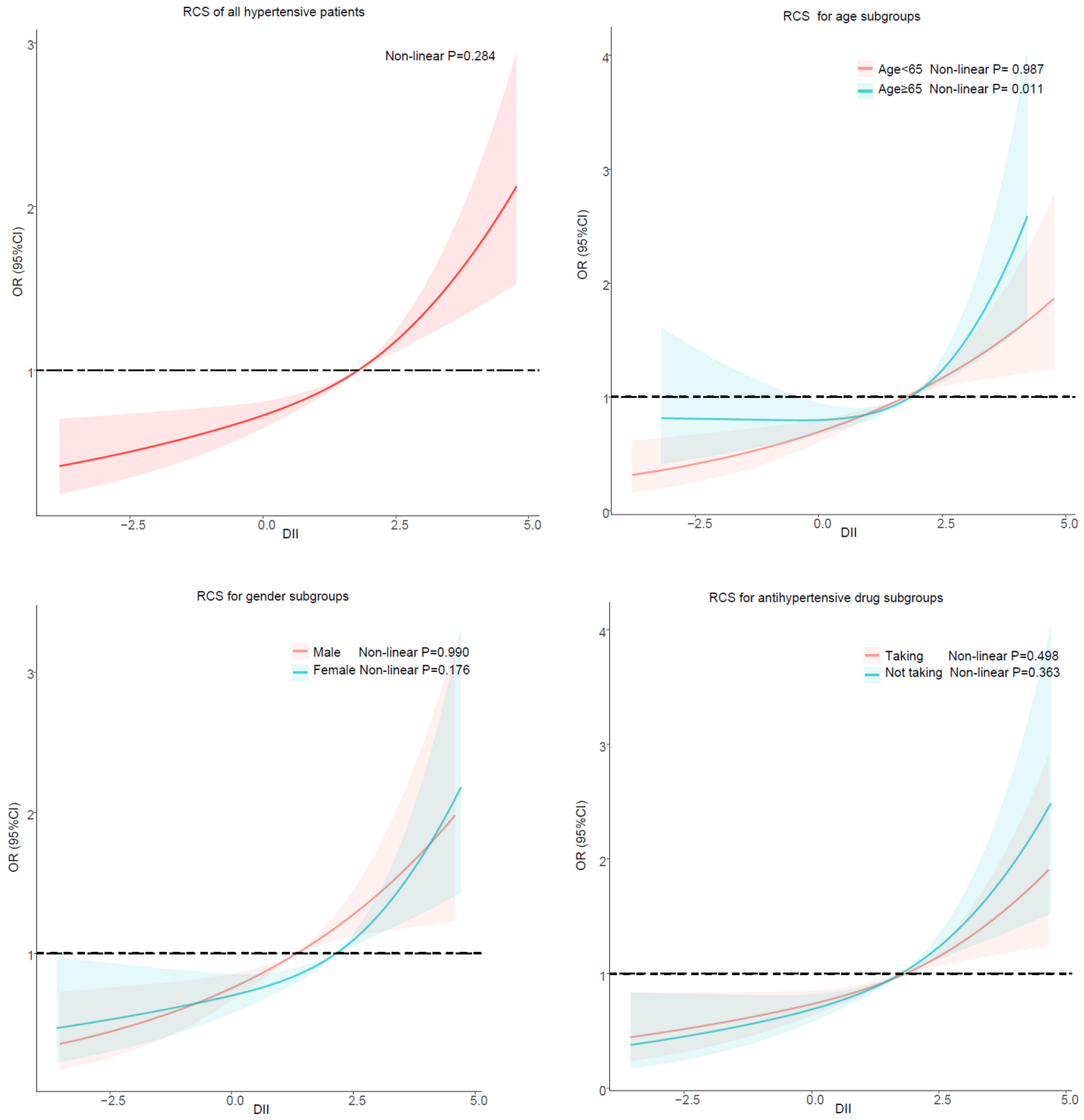

FIGURE 4
Potential nonlinear relationship between DII and sarcopenia (weighted).

Inflammation is one of the important ways of the occurrence and development of sarcopenia (38). Chronic inflammation can accelerate protein breakdown and promotes sarcopenia by activating the ubiquitin-proteasome system, caspase 3, lysosome, and myostatin (39). In addition, as stated in the preface, hypertension is currently recognized as an inflammation-related disease, and multiple inflammatory markers have been shown to be abnormally elevated in hypertensive patients (3–5), Activation of these inflammatory markers promotes sarcopenia through NF-κB and NLRP3 (6, 7). This provides a theoretical basis for preventing sarcopenia by regulating the inflammation level in hypertensive patients.

Diet is an effective measure to improve systemic inflammation. Studies have constructed DII based on the anti-inflammatory and pro-inflammatory levels of foods to assess dietary inflammatory potential (17). Evidence has demonstrated that the DII is significantly associated with various markers of systemic inflammation, such as C-reactive protein (CRP), neutrophil-to-lymphocyte ratio (NLR), tumor necrosis factor-alpha (TNF-α), and procalcitonin (40, 41). Nilufal Shoei et al. found that high DII is associated with an increased risk of hypertension (42). In addition, Cao et al. found that DII was associated with the risk of all-cause mortality in hypertensive patients, and the higher the DII, the higher the all-cause mortality in hypertensive patients (43). Our study links DII to sarcopenia in hypertensive patients. The results showed that the higher the DII, the higher the risk of sarcopenia in hypertensive patients. Previous studies have shown that DII was associated with sarcopenia in the elderly, CKD patients, and

Crohn's patients. The higher the DII, the higher the risk of sarcopenia (18, 19, 44). The results of these studies are consistent with ours.

Our results demonstrate that DII is significantly correlated with the risk of sarcopenia in hypertensive patients. Those with higher DII scores are more likely to suffer from sarcopenia. DII is an index to evaluate the dietary inflammatory potential of patients. Pro-inflammatory diet may promote the occurrence and development of myopathy by aggravating systemic inflammation levels in hypertensive patients to activate a variety of enzyme systems to accelerate muscle breakdown (39, 45). However, due to the cross-sectional nature of this study, we can only make this assumption, and further prospective investigations are needed to verify our hypothesis.

In subgroup analyses stratified by age, gender and antihypertensive drug, the result of regression analysis was in line with the primary findings. High levels of DII was an independent risk factor for sarcopenia in hypertensive patients. Our results are consistent with previous studies (46, 47). We further examined the potential nonlinear correlation between DII and sarcopenia by using restricted regression cubic splines. No potential non-linear relationship was observed in the RCS stratified by gender and antihypertensive medications. However, a non-linear connection between DII and sarcopenia in individuals aged 65 or above was observed in our findings. Too low DII had no significant preventive effect on patients with sarcopenia. It's not impossible to explain. DII was calculated and briefly explained as follows: DII for each nutrient or dietary ingredient = [(daily intake of that nutrient or dietary ingredient -global *per capita* daily intake of that nutrient or dietary ingredient)/that nutrient or dietary ingredient Standard deviation of global *per capita* daily intake] x inflammatory effect index of that nutrient or dietary ingredient, and the sum of DII of each nutrient or dietary ingredient was the total DII score of individual study subjects (26). Patients with low DII scores also have low intakes of various dietary substances (including energy, protein, fat, etc), which can lead to malnutrition (48, 49), a high risk factor for sarcopenia (50–52). Older people are known to be at high risk for malnutrition (53), so we speculate that in elderly patients with low DII, the risks of malnutrition may mask the benefits of an anti-inflammatory diet. But further prospective studies are needed to confirm our suspicions.

In this cross-sectional study, our results suggest that pro-inflammatory diet is an independent risk factor for sarcopenia in hypertensive patients. But consider that low DII scores are associated with the intake of various nutrients, which can lead to malnutrition and an increased risk of sarcopenia. Therefore, people at nutritional risk, such as the elderly, should pay attention to the intake of energy, protein, fat and other substances while maintaining an anti-inflammatory diet to prevent sarcopenia.

Limitations

There were some study limitations. First, it was subject to the limitations inherent of retrospective analysis. The relationship between DII and sarcopenia could only be interpreted as a correlation, rather than as a causal relationship. Second, Previous studies calculated DII based on 45 foods. Since only 28 dietary data from NHANES could be used to calculate DII, our study calculated the sum of DII for only 28 foods. However, previous studies have confirmed that DII calculated using only 28 foods does not affect the predictive effectiveness of DII (54). Third, Due to the limited data on grip strength in NHANES, the diagnosis of sarcopenia in our study relied solely on muscle mass without combining grip strength. Further prospective studies are needed to confirm our results.

Conclusion

DII is associated with the risk of sarcopenia in hypertensive patients. The higher the DII score, the higher the risk of sarcopenia. Low DII may not have a positive effect on the prevention of sarcopenia in hypertensive individuals older than 65 years.

Data availiability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Author contributions

LC designed the research and is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. JT conducted the analysis and wrote the first draft of the paper. SS, YuL, JX, YZ, BW, YiL, KC, GL, and LC revised the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

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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Prevalence and mortality risk of low skeletal muscle mass in critically ill patients: an updated systematic review and meta-analysis

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Background: Patients with critical illness often develop low skeletal muscle mass (LSMM) for multiple reasons. Numerous studies have explored the association between LSMM and mortality. The prevalence of LSMM and its association with mortality are unclear. This systematic review and meta-analysis was performed to examine the prevalence and mortality risk of LSMM among critically ill patients.

Methods: Three internet databases (Embase, PubMed, and Web of Science) were searched by two independent investigators to identify relevant studies. A random-effects model was used to pool the prevalence of LSMM and its association with mortality. The GRADE assessment tool was used to assess the overall quality of evidence.

Results: In total, 1,582 records were initially identified in our search, and 38 studies involving 6,891 patients were included in the final quantitative analysis. The pooled prevalence of LSMM was 51.0% [95% confidence interval (CI), 44.5–57.5%]. The subgroup analysis showed that the prevalence of LSMM in patients with and without mechanical ventilation was 53.4% (95% CI, 43.2–63.6%) and 48.9% (95% CI, 39.7–58.1%), respectively (P-value for difference = 0.44). The pooled results showed that critically ill patients with LSMM had a higher risk of mortality than those without LSMM, with a pooled odds ratio of 2.35 (95% CI, 1.91–2.89). The subgroup analysis based on the muscle mass assessment tool showed that critically ill patients with LSMM had a higher risk of mortality than those with normal skeletal muscle mass regardless of the different assessment tools used. In addition, the association between LSMM and mortality was statistically significant, independent of the different types of mortality.

Conclusion: Our study revealed that critically ill patients had a high prevalence of LSMM and that critically ill patients with LSMM had a higher risk of mortality than those without LSMM. However, large-scale and high-quality prospective cohort studies, especially those based on muscle ultrasound, are required to validate these findings.

Systematic review registration: http://www.crd.york.ac.uk/PROSPERO/, identifier: CRD42022379200.

KEYWORDS

prevalence, mortality, low skeletal muscle mass, critically ill patients, systematic review and meta-analysis

1. Introduction

The intensive care unit (ICU), created in 1953, has become an integral part of the health care system worldwide for patients with critical illness (1). The survival rates of patients with critical illness have improved because of major progress in medical technology, greater understanding of disease pathophysiology, and use of multidisciplinary approaches to care (2). However, improving the prognosis of critically ill patients remains an important issue for critical care departments. Numerous risk factors are closely related to the mortality of critically ill patients, such as undernutrition (3), multiple organ failure (4), advanced age (5), sepsis (6), muscle wasting, frailty (7), and other factors. Among these factors, muscle wasting has drawn increasing attention from intensive care physicians.

Muscle wasting, also termed myopenia, is defined as wasting or thinning of muscle mass (8). Muscle wasting is assessed using a semiautomatic method of measuring the amount of muscle area on a computed tomography (CT) scan using predefined Hounsfield units (9). The skeletal muscle index is then computed by dividing the estimated muscle area by the body height. A reasonable threshold for prediction of low skeletal muscle mass (LSMM) has been suggested to be a skeletal muscle index in the fifth age-matched percentile (10). Previous studies have confirmed that older people with muscle wasting have a high risk of falls (11), mortality (12), fractures (13), and functional decline (14), which lead to adverse impacts on the economy and society as well as increased healthcare expenditures (15). Therefore, management of LSMM has become an important issue. Numerous methods have been adopted to assess muscle mass, including magnetic resonance imaging, CT, ultrasound, dual-energy X-ray, bioelectrical impedance, and anthropometric methods (16). The most commonly used method in critically ill patients is CT.

In recent decades, LSMM has become a focus of research in critical care. Critically ill patients can easily develop LSMM secondary to malnutrition, inactivity, and inflammatory reactions (17). Several studies have explored the association between LSMM and adverse outcomes among critically ill patients (18–20). These studies showed that the presence of LSMM based on CT scans was associated with a high risk of all-cause death among critically ill patients (18, 21) and that measurement of the total psoas muscle area can improve the prediction of mortality (22). In addition, many studies have shown that the prevalence of LSMM among critically ill people is higher than that among older people (23, 24). A recent systematic review revealed an LSMM prevalence of 50.9% (25). However, this review consisted of only 9 studies involving 1,563 patients, and the studies used only CT to assess muscle mass. Some studies have detected LSMM by newer technologies such as ultrasound (26, 27). Most importantly, the above-mentioned systematic review did not perform a subgroup analysis. Moreover, many new articles have explored the impact of LSMM on mortality (18, 19, 21, 28–35). Therefore, we considered it very important to perform an updated systematic review to summarize the prevalence and mortality risk of LSMM in critically ill patients. The aim of our study was to systematically summarize the prevalence of muscle wasting among critically ill patients and identify whether critical illness with LSMM can increase the risk of mortality.

2. Methods

2.1. Search strategy

This systematic review is reported in accordance with the PRISMA guidelines and was preregistered in the PROSPERO database (CRD42022379200). Two authors searched for relevant articles in three internet databases (PubMed, Embase, and Web of Science) from database inception to 1 September 2022. We used the following keywords and Medical Subject Headings (MeSH) terms to identify relevant studies: "muscle mass" or "muscle wasting" or "low skeletal muscle" and ("mortality" or "death" or "survival") and "critically ill patient". The detailed search strategy is shown in Supplementary File 1.

2.2. Inclusion and exclusion criteria

The patients, intervention, comparison, outcomes, and study design (PICOS) principle was adopted to confirm study eligibility. The inclusion criteria were as follows: (1) the patients involved in the study were critically ill (i.e., adult patients treated in the ICU); (2) as the exposure, LSMM was definitively diagnosed based on CT scans, anthropometric methods, and ultrasound; (3) the article presented the prevalence of LSMM, or the prevalence could be calculated using the data available within the article; and (4) the study design was observational (cohort study or cross-sectional study). Reviews, case reports, comments, correspondence articles, letters, and abstracts were excluded because complete quality assessment of such reports could not be performed.

2.3. Study selection and data extraction

Two authors independently formulated the search strategy and screened the articles. First, the results of the relevant studies from the three databases were imported into EndNote X9 software, and duplicates were deleted. Next, the authors screened the title and abstract based on the PICOS principle, checked the abstract for potential relevance, and screened the full text. The final studies were confirmed after careful review of the full text. During this process, disagreements were resolved by discussion with a third reviewer. Two authors also independently extracted the data based on standardized forms consisting of author, year, country, main diagnosis, age, sample size, prevalence of muscle wasting, number of female/male participants, prevalence of muscle wasting by sex, muscle wasting assessment tool used, and effect size of the association between LSMM and mortality.

2.4. Assessment of study quality

We used the Newcastle–Ottawa Scale to assess the quality and methodology of the included studies. The assessing item including selection, comparability, and outcome. To minimize the potential for bias, we had two reviewers independently evaluate each included study using the Newcastle-Ottawa Scale, and we

resolved any discrepancies through discussion and consensus. The total score of the included studies ranged from 0 to 9 points, and the quality of the study was defined as poor, moderate, or high with corresponding scores of 0–4, 5–6, and 7–9 points, respectively.

2.5. The quality of evidence

We used GRADE tool to assess the overall quality of the evidence. This tool consisted of five items including risk of bias, inconsistency, indirectness, imprecision and publication bias.

2.6. Outcome measures

The primary outcome of this systematic review was the prevalence of LSMM, and the secondary outcome was all-cause mortality.

2.7. Statistical analysis

All statistical analyses were performed with Stata Version 14 (StataCorp, College Station, TX, USA). Metaprop, a Stata command, was used to pool the prevalence of muscle wasting from each included study, and the metan command was used to combine the results regarding the association between LSMM and mortality risk of all studies. A random-effects model was used because of the high heterogeneity ($I^2 > 50\%$) across studies caused by differences in countries, definitions, sample sizes, and reasons for ICU admission. In addition, to detect the original cause of heterogeneity, different subgroup analyses based on country, sex, sample size (<100 vs. ≥ 100), age group, main diagnosis for ICU admission, mechanical ventilation, and type of outcome were performed if there were more than two studies within each stratum. Finally, a sensitivity analysis and test of publication bias were performed.

3. Results

3.1. Study selection

In total, 1,582 records were identified from 3 databases (PubMed, $n = 615$; Embase, $n = 743$; and Web of Science, $n = 224$). After deleting duplicates, 1,357 studies remained to be screened. Two authors deleted 1,269 studies after checking the title and abstract, resulting in 88 studies for full-text review. Of these, 38 studies were included in the final quantitative analysis based on the inclusion and exclusion criteria (18–21, 24, 26–58). The main reasons for exclusion were an ineligible study design and irrelevant studies exploring the association between LSMM and other clinical outcomes (Figure 1).

3.2. Characteristics of included studies

Thirty-eight studies involving 6,891 participants met the eligibility criteria. The participants' mean or median age ranged from 41.4 to 79 years. Most of the studies were conducted in the United States ($n = 9$), followed by Korea ($n = 7$) and Japan ($n = 6$). Four studies were performed in the Netherlands, three in China, and two in Italy. Only one study each was conducted in Brazil, India, Germany, and Malaysia. The main diagnosis among the participants in the included studies was sepsis (14 studies), trauma (7 studies), surgical diseases (3 studies), and COVID-19 (1 study). Thirteen studies involved patients with mixed diagnoses collectively referred to as "critical illness". The highest prevalence of LSMM was 90%, and the lowest prevalence was 25%. The largest sample size of the included studies was 905 (33), and the smallest sample size was 37 (31). A total of 89.4% of studies used CT scans to assess muscle mass, whereas only two studies used ultrasonography (26, 27) and two used anthropometric methods (19, 50). Thirty studies explored the association between LSMM and mortality among critically ill patients (Table 1). Thirteen studies considered the in-hospital mortality as the main outcome and six studies reported 30-day mortality, followed 6 studies for 1-year mortality. Supplementary Table 1 displayed the time for assessing muscle mass.

3.3. The diagnosis criteria and cut-off points for LSMM of each study

Based on the information provided, the diagnosis criteria and cut-off points for LSMM among the included studies were summarized in Supplementary Table 2. For CT-scans, the majority of studies used skeletal muscle mass index (SMI) to define LSMM. Some studies used skeletal muscle area (SMA), and only two studies adopted total psoas area (TPA). In addition, ultrasonography was used in some studies to assess the Femoris Muscle for confirming LSMM. Whereas, the cut-off value for confirming LSMM were varied across these studies.

3.4. Meta-analysis of prevalence of LSMM in critically ill patients

In total, 37 studies reported the prevalence of LSMM among critically ill patients (24, 27–33, 36–52). The prevalence ranged from 25 to 90%, and the pooled prevalence of LSMM was 51.0% [95% confidence interval (CI), 44.5–57.5%] (Figure 2).

3.5. Subgroup analyses of pooled prevalence by different variables

3.5.1. Subgroup analysis by region

The results of the subgroup analysis of the pooled prevalence of LSMM based on region showed that the prevalence of LSMM was 51.1% (95% CI, 40.6–61.6%) among Asians, 46.9% (33.9–60.0%)

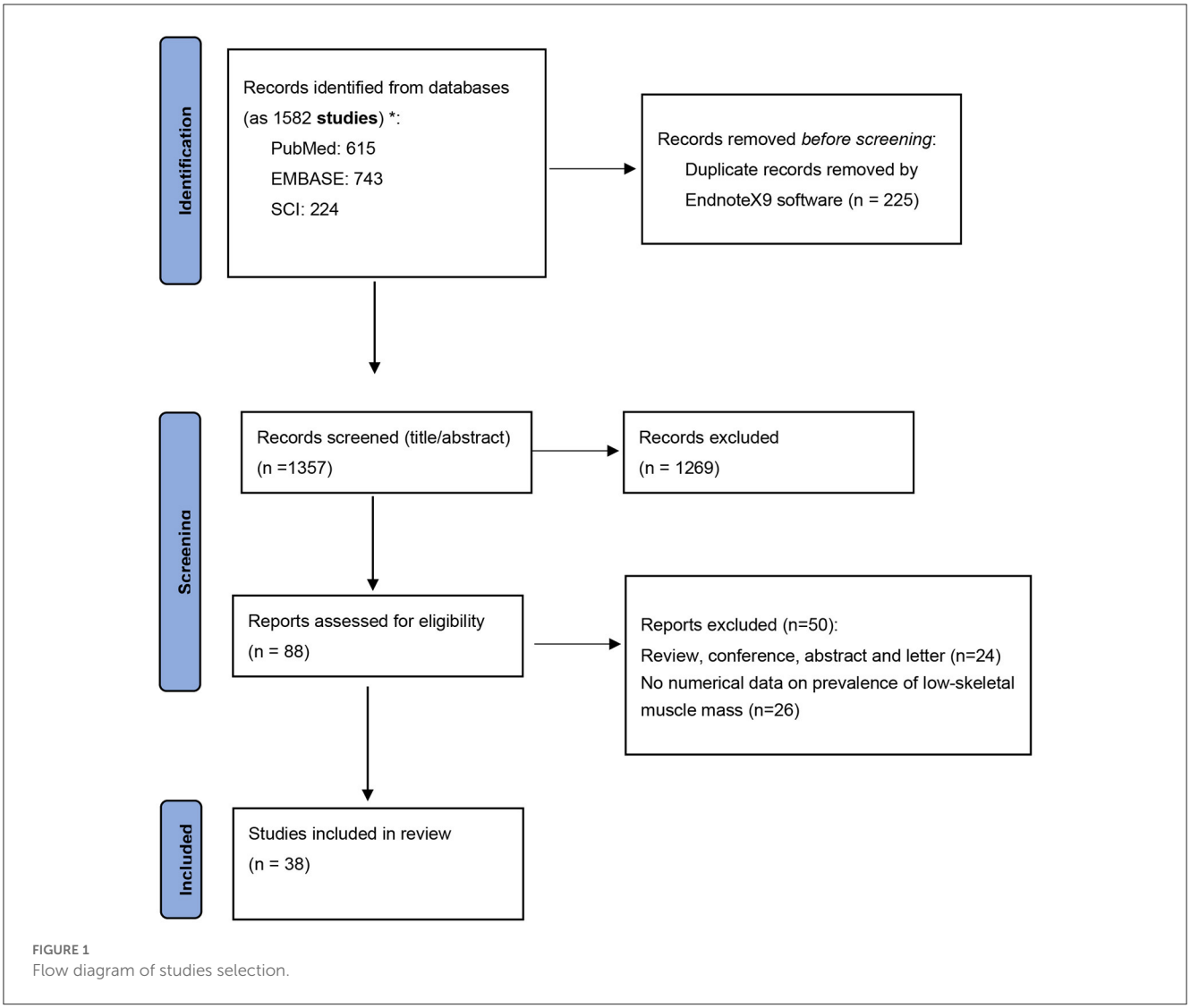

FIGURE 1
Flow diagram of studies selection.

among Europeans, 51.7% (43.2–60.2%) among Americans, and 65.8% (60.6–70.9%) among Oceanians (Table 2).

3.5.2. Subgroup analysis by sex

Twenty-five studies provided the prevalence of LSMM by sex. The results showed no statistically significant difference in the prevalence of LSMM based on sex. The prevalence of LSMM was 48.8% (95% CI, 40.0–57.6%) among men and 45.5% (37.9–53.2%) among women (Table 2).

3.5.3. Subgroup analysis by mechanical ventilation

Seventeen studies included critically ill patients who underwent mechanical ventilation during the hospitalization period. The results of this subgroup analysis showed that the prevalence of LSMM was slightly higher in patients with than without mechanical ventilation, at 53.4% (43.2–63.6%) and 48.9% (39.7–58.1%), respectively. However, there was no significant difference between these two groups ($P = 0.44$; Table 2).

3.5.4. Subgroup analysis by diagnosis

We categorized the studies into four classifications based on the main diagnosis. Fourteen studies focused on sepsis, and the prevalence of LSMM among these participants was 55.1% (95% CI, 44.6–65.6%). Seven studies focused on critically ill patients with trauma, and the prevalence of LSMM was 47.6% (33.8–61.4%). Only three studies focused on surgical patients, among whom the prevalence of LSMM was 43.0% (24.4–61.6%). The remaining 13 studies involved patients with mixed diagnoses, and the pooled prevalence of LSMM was 50.2% (38.4–62.0%) (Table 2).

3.5.5. Other subgroup analyses of prevalence of LSMM

We split the sample size into two groups (<100 vs. ≥100), and the prevalence of LSMM was similar between the two groups at 49.2% (41.4–57.2%) and 51.8% (43.5–60.2%), respectively. Additionally, a subgroup analysis between age groups split by 60 years showed no statistically significant difference between these

TABLE 1 Characteristics of the included studies.

References	Country	Mechanical ventilation	Design	Age/mean (SD)/median	Main diagnosis	Total sample size	Prevalence of LSMM	Muscle mass assessment tool	Type of outcomes
Akahoshi et al. (36)	Japan	Yes	Retrospective study	49.95 ± 16.3	Trauma	84	0.30	CT	30-day mortality
Baggerman et al. (37)	Netherlands	No	Retrospective cohort study	66.0 ± 13.6	Sepsis	155	0.31	CT	In-hospital mortality
Bareto (38)	USA	No	Retrospective cohort study	63.4 ± 16.3	Sepsis	81	0.70	CT	NA
Cho et al. (39)	Korea	Yes	Retrospective cohort study	≥18	Critically ill	127	0.37	CT	1-year mortality
Cox et al. (28)	USA	No	Prospective cohort study	53 ± 14	Sepsis	47	0.49	CT	30-day mortality
Damanti et al. (40)	Italy	Yes	Cross-sectional	59.3 ± 11.91	COVID-19	81	0.65	CT	NA
Ebbeling et al. (42)	USA	Yes	Prospective study	74 ± 3.17	Trauma	180	0.50	CT	In-hospital mortality
de Hoogt et al. (41)	Netherlands	No	Retrospective cohort study	None	Critically ill	139	0.32	CT	In-hospital mortality
Hwang et al. (43)	USA	No	Retrospective cohort study	63.7 ± 16.4	Critically ill	230	0.32	CT	In-hospital mortality
Ji et al. (44)	China	Yes	Retrospective study	68.75 ± 4.17	Sepsis	236	0.48	CT	30-day mortality
Joyce et al. (24)	Australia	Yes	Retrospective study	63.7 ± 16.4	Sepsis	279	0.68	CT	30-day mortality
Ju et al. (45)	Korea	Yes	Prospective study	64.3 ± 11.2	Critically ill	125	0.90	CT	NA
Kaplan et al. (46)	USA	No	Retrospective cohort study	None	Trauma	450		CT	1-year mortality
Khan et al. (29)	India	Yes	Prospective study	48.37 ± 11.29	Critically ill	111	0.68	CT	ICU-mortality
Kim et al. (30)	Korea	No	Case-control	≥18	Sepsis	516	0.82	CT	1-year mortality
Koga et al. (47)	Japan	No	Retrospective study	≥18	Sepsis	191	0.48	CT	In-hospital mortality
Kou et al. (48)	China	Yes	Retrospective study	68.75 ± 4.17	Surgery	96	0.31	CT	In-hospital mortality
Looijaard et al. (49)	Netherlands	No	Prospective study	59 ± 17	Critically ill	110	0.47	CT	NA
Malle et al. (31)	Australia	No	Retrospective study	58.8 ± 17.3	Critically ill	37	0.49	CT	6-month Mortality
Moisey et al. (51)	USA	Yes	Retrospective cohort study	79 ± 2.7	Trauma	149	0.71	CT	In-hospital mortality
Moon et al. (32)	Korea	Yes	Retrospective study	78 ± 1.33	Sepsis	190	0.51	CT	In-hospital mortality

(Continued)

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TABLE 1 (Continued)

References	Country	Mechanical ventilation	Design	Age/mean (SD)/median	Main diagnosis	Total sample size	Prevalence of LSMM	Muscle mass assessment tool	Type of outcomes
Loosen et al. (58)	Germany	Yes	Retrospective cohort study	60 (21–88)	Critically ill	155	NA	CT	1-year mortality
Lucidi et al. (50)	Italy	No	Retrospective study	49.7 ± 16	Sepsis	74	0.43	Anthropometric	In-hospital mortality
Mueller et al. (27)	USA	No	Prospective study	61.9 ± 15.8	Critically ill	102	0.43	Ultrasound	In-hospital mortality
Ng et al. (52)	Malaysia	Yes	Retrospective study	54.4 ± 17.8	Critically ill	228	0.50	CT	In-hospital mortality
Oh et al. (33)	Korea	No	Retrospective study	65.7 ± 15.0	Sepsis	905	0.45	CT	1-years mortality
Okada et al. (34)	Japan	No	Retrospective study	76 (64–84)	Sepsis	255	0.33	CT	90-day mortality
Proksch et al. (35)	USA	No	Prospective cohort study	70	Trauma	76	0.50	CT	6-month mortality
Seo et al. (53)	Korea	No	Retrospective study	65.0 (58.0–72.0)	Sepsis	175	0.86	CT	30-day mortality
Sheean et al. (54)	USA	Yes	Cross-sectional	59.2 ± 15.6	Sepsis	56	0.61	CT	NA
Shibahashi et al. (55)	Japan	No	Retrospective cohort study	75 (68–82)	Sepsis	150	0.55	CT	In-hospital mortality
Shibahashi et al. (56)	Japan	No	Retrospective cohort study	>60	Trauma	74	0.54	CT	NA
Toledo et al. (20)	Brazil	No	Retrospective cohort study	61.6 ± 13.5	Critically ill	99	0.38	CT	30-day mortality
Vongchaiudomchoke et al. (19)	Thailand	Yes	Prospective study	75.0 ± 7.6	Surgery	120	0.33	Anthropometric	120-day mortality
Weijs et al. (57)	Netherlands	Yes	Retrospective study	59.5 ± 17.8	Critically ill	240	0.63	CT	In-hospital mortality
Woo et al. (18)	Korea	Yes	Retrospective study	66.4 ± 14.5	Surgery	45	0.67	CT	NA
Xi et al. (21)	China	Yes	Retrospective study	41.4 ± 15.9	Trauma	451	0.25	CT	NA
Yanagi et al. (26)	Japan	No	Prospective cohort study	70 (60–76)	Critically ill	72	0.36	Ultrasound	1-year mortality

CT, computed tomography scan; NA, not available.

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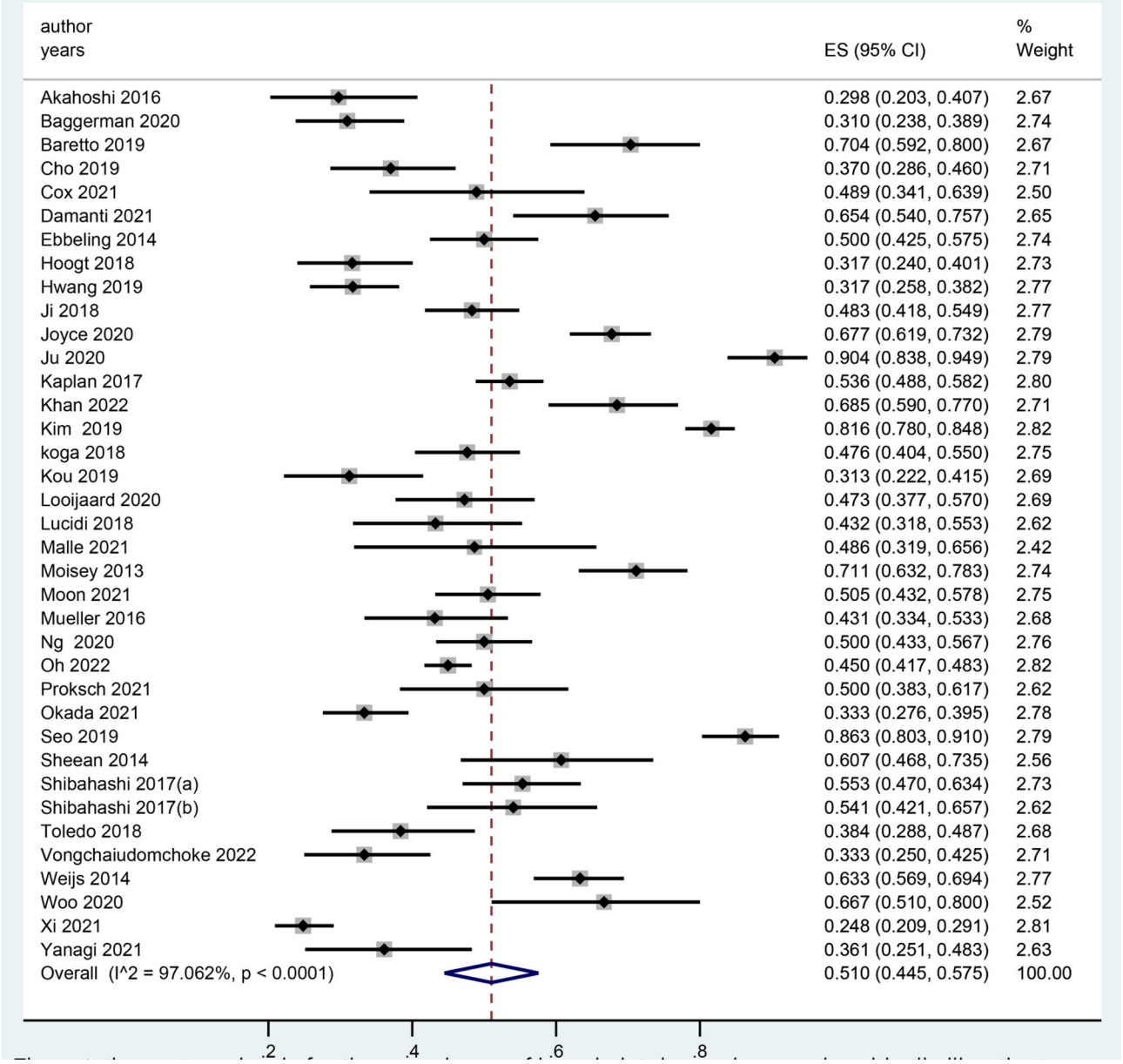

FIGURE 2
The meta-anlaysis for the prevalence of low skeletal muscle mass in critically ill patients.

two age groups (51.5%, 95%CI: 42.7–60.4%; vs. 50.0%, 95%CI: 38.9–61.0%) (Table 2).

3.6. Meta-analysis of association between LSMM and mortality risk

Thirty studies explored the association between LSMM and mortality risk (19, 20, 24, 26–37, 39, 41–44, 46–48, 50–53, 55, 57, 58). The pooled odds ratio (OR) for the association between LSMM and mortality risk was 2.35 (95% CI, 1.91–2.89), which indicated that critically ill patients with LSMM had an approximately 2.35 higher likelihood of mortality than those without LSMM (Figure 3).

3.7. Subgroup analysis of association between LSMM and mortality risk by different variables

3.7.1. Subgroup analysis based on mechanical ventilation

For critically ill patients who were mechanically ventilated, the pooled OR for the association between LSMM and mortality risk

TABLE 2 Subgroup analyses for the meta-analysis of prevalence of LSMM among critically ill patients.

Variables	Number of studies	Prevalence	95%CI	I^2	*P*-value for difference
Country					0.72
Asian	19	51.1%	40.6–61.6%	98.21%	
Europeans	6	46.9%	33.9–60.0%	93.23%	
Americas	10	51.7%	43.2–60.2%	90.64%	
Oceanias	2	65.8%	60.6–70.9%	97.06%	
MV					0.44
Yes	17	53.4%	43.2–63.6%	97.14%	
No	20	48.9%	39.7–58.1%	97.28%	
Gender					0.51
Male	25	48.8%	40.0–57.6%	96.75%	
Female	25	45.5%	37.9–53.2%	92.80%	
Diagnose					0.65
Trauma	7	47.6%	33.8–61.4%	96.53%	
Sepsis	14	55.1%	44.6–65.6%	97.59%	
Mixed diagnoses	13	50.2%	38.4–62.0%	96.51%	
Surgery	3	43.0%	24.4–61.6%	0%	
Muscle mass measurement					0.01
CT	33	52.5%	45.5–59.4%	97.29%	
Anthropometric methods	2	36.9%	30.1–43.6%	0%	
Ultrasonography	2	40.1%	32.9–47.4%	0%	
Sample size					0.63
≥100	24	51.8%	43.5–60.2%	97.97%	
<100	13	49.2%	41.4–57.2%	84.47%	
Age group					0.79
≥60	21	51.5%	42.7–60.4%	97.0%	
<60	11	50.0%	38.9–61.0%	94.8%	

CT, computed tomography scan.

was 2.32 (95% CI, 1.85–2.90). Critically ill patients with LSMM who were not ventilated also had a high risk of mortality, with a pooled OR of 2.42 (95% CI, 1.74–3.36) (Table 3).

3.7.2. Subgroup analysis based on outcomes

Critically ill patients with LSMM had a higher risk of mortality than those with normal skeletal muscle mass regardless of the type of outcome (in-hospital mortality, 30-day mortality, or 1-year mortality) with an OR of 2.27 (1.71–3.01), 3.23 (1.54–6.75), and 2.60 (1.67–4.06), respectively.

3.7.3. Subgroup analysis based on assessment tools

We also performed a subgroup analysis based on the assessment tools used. The majority of the studies used CT for assessment of LSMM, and a few studies used anthropometric methods for defining LSMM; only two studies applied ultrasonography. The results showed that critically ill patients with LSMM had a higher mortality risk than those without LSMM when using CT or anthropometric methods; the pooled ORs were 2.25 (95% CI, 1.82–2.80) and 2.79 (1.36–5.74), respectively. We also found an independent association between LSMM and mortality risk among critically ill patients when using ultrasonography for confirmation (pooled OR,5.86; 95% CI, 1.89–18.20) (Table 3).

3.7.4. Subgroup analysis based on diagnosis

Critically ill patients with LSMM had a higher mortality risk than those without LSMM among participants with sepsis (pooled OR, 1.80; 95% CI, 1.56–2.09), surgery (pooled OR, 2.72; 95% CI, 1.30–5.70), or mixed diagnosis (pooled OR, 3.21; 95% CI, 2.01–5.13). However, this association was not statistically significant among trauma patients (pooled OR, 2.14; 95% CI, 0.79–5.83) (Table 3).

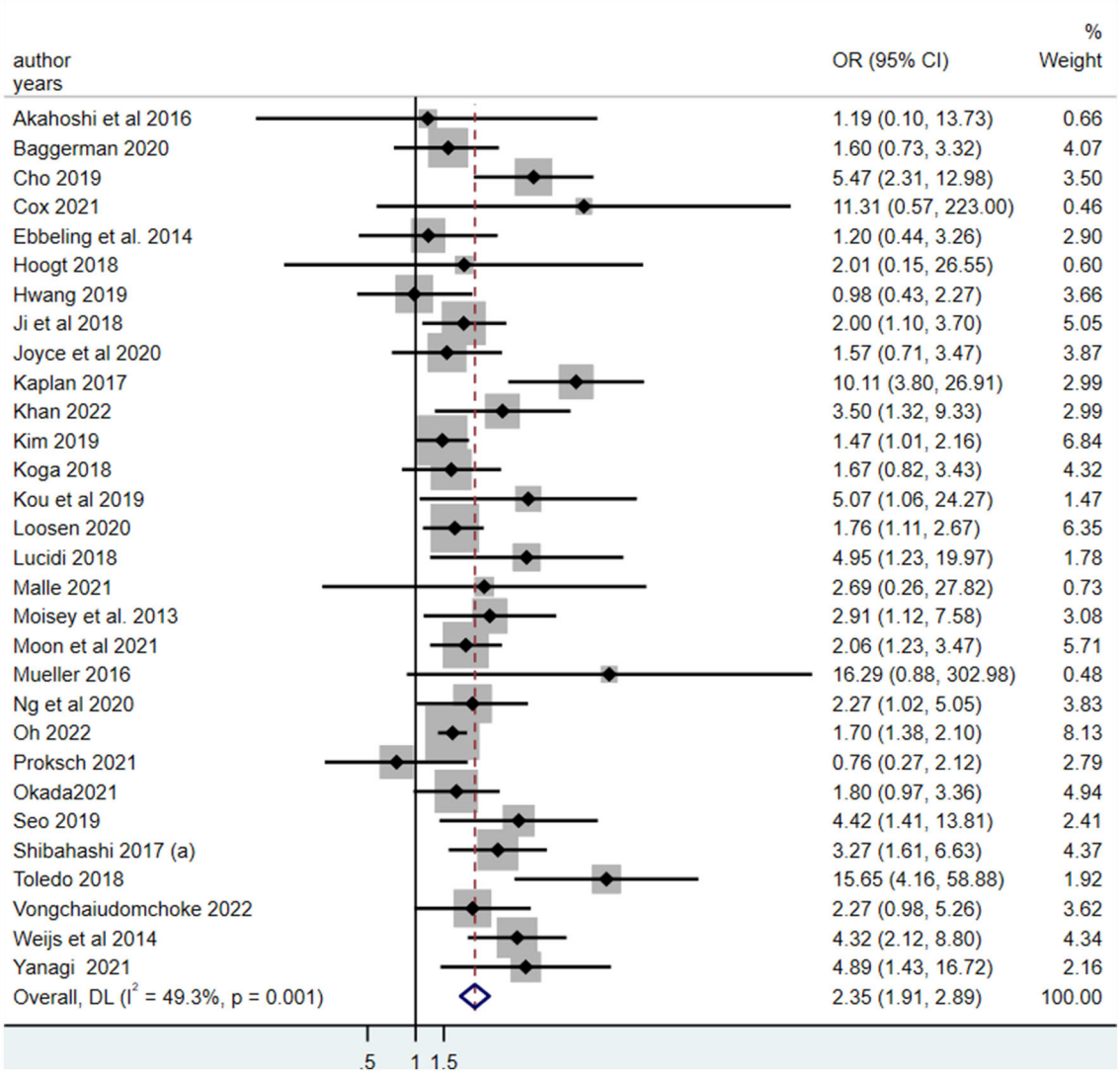

FIGURE 3
The meta-analysis for the association between the low skeletal muscle mass and all-cause mortality in critically ill patients.

3.7.5. Other subgroup analyses of association between LSMM and mortality risk by country and sample size

We also performed subgroup analyses based on country and sample size. These results also showed that critically ill patients with LSMM had a higher mortality risk than their counterparts without LSMM, and the results were independent of country and sample size (Table 3).

3.8. Publication bias

We tested the publication bias for the outcomes of the prevalence of LSMM and mortality. The results showed no potential publication bias when pooling the prevalence of LSMM as indicated by Begg's test (*P*-value = 0.27) (Supplementary Figure 1a). However, the results of Begg's test (*P*-value = 0.05) showed potential publication bias when the results for the association between LSMM and mortality risk were pooled (Supplementary Figure 1b).

3.9. Quality assessment

The Newcastle–Ottawa Scale score ranged from 5 to 8 points, and most of the studies had 7 points. This result indicated that the quality of the included studies was relatively good (Supplementary Table 3).

TABLE 3 Subgroup analyses of the association between low skeletal muscle mass and mortality in critically ill patients.

Variables	Number of studies	OR	95%CI	I^2	*P*-value for difference
Country					0.71
Asian	15	2.14	1.75–2.61	26.1%	
Europe	5	2.40	1.48–3.87	37.9%	
Americas	8	3.19	1.31–7.76	76.6%	
Oceania	2	1.66	0.78–3.52	0%	
MV					0.47
Yes	13	2.32	1.85–2.90	10.4%	
No	17	2.42	1.74–3.36	61.5%	
Diagnose					0.11
Trauma	5	2.14	0.79–5.83	74.0%	
Sepsis	12	1.80	1.56–2.09	0%	
Mixed diagnosis	11	3.21	2.01–5.13	56.9%	
Surgery	2	2.72	1.30–5.70	0%	
Muscle mass measurement					0.26
CT	26	2.25	1.82–2.80	51.4%	
Anthropometric methods	2	2.79	1.36–5.74	0%	
Ultrasonography	2	5.86	1.89–18.20	0%	
Sample size					0.21
≥100	22	1.95	1.72–2.21	54.7%	
<100	8	3.38	1.99–5.74	44.5%	
Type of outcomes					0.56
30-day mortality	6	3.23	1.54–6.75	56.4%	
In-hospital mortality	13	2.27	1.71–3.01	22.7%	
1-year mortality	6	2.60	1.67–4.06	77.4%	
Others	5	1.89	1.19–2.99	17.1%	

CT, computed tomography scan.

3.10. The results of overall quality of evidence

The summary results of GRADE were showed in Supplementary Table 4, indicating that the evidence was low because there were few inconsistencies in some included studies and they were some publication bias across these studies.

3.11. Sensitivity analysis

A sensitivity analysis was performed by omitting one study and pooling the remaining studies to determine whether the pooled results showed major changes. The results of the sensitivity analysis regarding prevalence or mortality showed no significant changes (Supplementary Figure 2). In addition, we also conducted sensitivity analysis by omitting the studies that used anthropometric methods to define LSMM and the results was almost not changed, which indicated the results was stable (Supplementary Figure 3).

4. Discussion

Our study showed that the prevalence of LSMM among critically ill patients was very high at 51.0% (95% CI, 44.5–57.5%), meaning that more than half of critically ill patients had LSMM. This study also indicated that critically ill patients with LSMM had an approximately 2.35-fold higher mortality risk than those without LSMM. This systematic review and meta-analysis suggests that greater attention to LSMM, early screening of patients at high risk of LSMM, and timely interventions such as comprehensive treatments consisting of exercise and nutrition programs must be performed to slow down the process of muscle wasting. These efforts might reduce the mortality rate among critically ill patients.

Critically ill individuals often lose muscle mass for multiple reasons (17), such as extended time of inactivity, nutrient deficits, and impaired equilibrium between muscle protein synthesis and

breakdown. In 2021, a systematic review of nine studies explored the association between LSMM defined by CT and mortality among critically ill patients (25). That study indicated that LSMM based on CT was associated with high short-term mortality. Notably, the study had two main limitations. First, their search strategy was relatively old studies, resulting in only 9 studies involving 1,563 patients, which might have influenced the representativeness of their findings. Second, the authors did not perform subgroup analyses based on different variables; such analyses are very important. Given that more researchers and clinicians are paying attention to LSMM in critically ill patients, increasing numbers of studies are focusing on this topic. Therefore, an updated meta-analysis that can provide an overall picture of the impact of LSMM on critical illness is required. Our study has overcome these limitations.

The pooled prevalence of LSMM in our study was very high at 51.0%. A previous systematic review showed that the pooled prevalence of LSMM was 46.2% (95% CI, 43.95–48.45%) among patients with metastasized colorectal cancer, which was very close to our results (59). We speculate that malnutrition, anorexia, and inflammatory reactions are common characteristics among patients with cancer and critically ill patients, leading to a high prevalence of LSMM (60). In addition, inactivity due to disease and treatment procedures places critically ill patients at high risk for LSMM. Therefore, early screening for LSMM among these select patients is very important.

The present study showed that critically ill patients undergoing mechanical ventilation might have a higher prevalence of LSMM than those without this treatment procedure, although there was no statistically significant difference. We speculate that LSMM may be worse in critically ill inpatients with than without mechanical ventilation, but this requires further study. Patients who require mechanical ventilation often have major lung disease. Mechanical ventilation prevents oral ingestion of food (61); instead, these patients require nasogastric tube feeding or other methods for nutrition and energy, which might not provide sufficient nutrition, thus reducing their protein intake. In addition, mechanical ventilation makes critically ill inpatients immobile. For these two main reasons, critical illness may be associated with a high prevalence of LSMM. Moreover, our study showed that the prevalence of LSMM among patients with sepsis was higher than that among patients with other diagnoses. The reason for this difference might be that patients with sepsis often have a high inflammatory response that leads to multiple organ disorders and malnutrition, resulting in a high risk of LSMM (62, 63). In addition, a previous study revealed that the mechanisms by which sepsis leads to LSMM are disorganized sarcomeres and myofibril dysfunction (64).

Our study also showed that patients with LSMM had a higher risk of mortality than those with normal muscle mass, and this result was in line with a previous study (25). LSMM is the main element in muscle wasting and has been widely confirmed to increase the risk of mortality across different populations (65–68). The mechanisms underlying the association between LSMM and mortality were elucidated in a previous study. In general, most critically ill patients with LSMM had serious comorbidities that increased their mortality risk. In addition, patients with LSMM may have decreased immune system function, which reduces their ability to resist the wide-ranging adverse effects of many treatment procedures such as mechanical ventilation, polypharmacy, and pulse index continuous cardiac output monitoring. Therefore, LSMM complicated by worsening of the patient's primary disease can become a vicious circle that results in a high likelihood of mortality.

Ultrasound measurement is a convenient, widely applied method with which to determine whether patients with critical illness have LSMM (69, 70). Multiple studies have compared the performance between B-mode ultrasound images and typical methods of defining LSMM (71–73). One study indicated that B-mode ultrasound imaging has great potential as a surrogate diagnostic tool for LSMM. Our study also showed a statistically significant association between LSMM and mortality, which is consistent with a previous study (74). One study in which ultrasound was used to detect muscle psoas indices showed that the psoas muscle index was associated with mortality, with a hazard ratio of 0.93 (95% CI, 0.876–0.987) (71). In addition, a study conducted in 2022 revealed that muscle thickness tested by ultrasound was independently associated with death among patients undergoing hemodialysis (73). Therefore, the association between LSMM based on ultrasound and mortality among critically ill patients should be further explored. Given the simplicity, practicality, and convenience of ultrasound for assessing muscle mass, we expect that this technique will provide prognostic value for predicting mortality among critically ill patients.

Our study had several strengths and limitations. First, compared with previous studies, this updated meta-analysis provided a more in-depth subgroup analysis of the pooled prevalence of LSMM and pooled the association between LSMM and mortality. The subgroup analysis based on diagnosis and measurement assessment for LSMM provides more valuable information that can guide clinical practice. Second, this meta-analysis included multiple studies involving 6,891 participants, thus providing a highly representative sample of this special population. Third, a comprehensive statistical analysis was used to test the robustness of our study. However, our meta-analysis also had some limitations. First, there was potential publication bias regarding the association between LSMM and mortality. Some non-English-language studies might have been excluded from the meta-analysis. Second, some studies used the results of a univariate analysis to assess the association between LSMM and mortality, potentially resulting in overestimation of the effect by confounding factors. Third, we considered the effect size by the hazard ratio to be equal to the OR in our study, and caution is therefore required when interpreting our main findings. Fourth, the overall quality of GRADE assessment was "low", more large-scale and high-quality prospective cohort studies, especially those based on muscle ultrasound, are required to validate these findings.

5. Conclusion

Our study showed a high prevalence of LSMM among critically ill patients and revealed that critically ill patients with LSMM had an 2.35-fold higher mortality risk than those with normal muscle mass. This study indicates that the importance of muscle mass as a potentially important prognostic factor in critically ill patients.

Healthcare providers were encouraged to carefully consider the risks and benefits of using CT for muscle mass measurement in critically ill patients, and to consider alternative methods such as ultrasound or bioelectrical impedance analysis (BIA) where appropriate. Early interventions, such as mobilizing patients and providing nutritional support, after confirming LSMM may reduce the mortality rate in this particular population.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding authors.

Author contributions

HY and X-XW were responsible for screening and extracting the studies. HM and ZL were responsible for study quality assessment. HY, LW, and X-MZ were responsible for statistical analysis. YX and X-MZ were responsible for the study design and review. HY, X-XW, and X-MZ drafted the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

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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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2023.1117558/full#supplementary-material

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The association of dietary inflammatory potential with skeletal muscle strength, mass, and sarcopenia: a meta-analysis

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Aims: Evidence suggested that dietary inflammatory potential may be associated with age-related skeletal muscle decline, but the results remained controversial. To summarize the evidence for the relationships between dietary inflammatory potential and skeletal muscle strength, mass, and sarcopenia in adults we conducted this meta-analysis.

Methods: Embase, Pubmed, and Web of Science were searched from inception up to 12 March 2023 for studies that evaluated the associations of dietary inflammatory potential [estimated by the Dietary inflammatory index (DII)] with skeletal muscle strength, mass, and sarcopenia. A meta-analysis was then performed to calculate the pooled regression coefficient (β) and odds ratio (OR). The non-linear dose-response relation between DII and sarcopenia was assessed using random-effects dose-response meta-analysis.

Results: This meta-analysis included 24 studies involving 56,536 participants. It was found that high DII was associated with low skeletal muscle strength [OR 1.435, 95% confidence interval (CI) 1.247–1.651, $P < 0.001$, $I^2 = 4.97\%$]. There was a negative association of DII with skeletal muscle strength (β −0.031, 95% CI −0.056 to −0.006, $P = 0.017$, $I^2 = 72.69\%$). High DII was also associated with low skeletal muscle mass (OR 1.106, 95% CI 1.058–1.157, $P < 0.001$, $I^2 = 0\%$). DII had a negative relationship with skeletal muscle mass with high heterogeneity (β −0.099, 95% CI −0.145 to −0.053, $P < 0.001$, $I^2 = 88.67\%$); we downgraded the inconsistency in the subgroup analysis of overweight/obese participants (β −0.042, 95% CI −0.065 to −0.019, $I^2 = 12.54\%$). Finally, the pooled results suggested that high DII was significantly associated with sarcopenia with significant heterogeneity (OR 1.530, 95% CI 1.245–1.880, $P < 0.001$, $I^2 = 69.46\%$); age and BMI may contribute partially to the heterogeneity since heterogeneity was decreased in the subgroup of older age (OR 1.939, 95% CI 1.232–3.051, $I^2 = 0\%$) and the group of overweight/obesity (OR 1.853, 95% CI 1.398–2.456, $I^2 = 0\%$). There was a non-linear dose-response association between DII and sarcopenia ($P = 0.012$ for non-linearity).

Conclusion: This meta-analysis suggested that higher dietary inflammatory potential was significantly associated with lower skeletal muscle strength, mass, and risk of sarcopenia. Future studies with consistent assessment and standardized methodology are needed for further analysis.

KEYWORDS

dietary inflammatory index (DII), sarcopenia, muscle, meta-analysis, nutrition

1. Introduction

Muscle strength plays a critical role in physical function, independence, and vitality in the aged (1, 2), and could predict subsequent health status of the older population and even the risk of mortality (3–7). Loss of muscle strength began at around the 30s and those in their 80s could lose up to 40% of their muscle strength compared with their 20s (8). Reduction of muscle mass, one of the hallmarks of aging, combined with the loss of muscle strength, is referred to as sarcopenia. Sarcopenia as an independent condition recognized by World Health Organization (9), was reported to affect 10% to 27% of people older than 60 years globally and the number of individuals affected by this condition was deemed to increase with the population aging (10). However, a clear understanding of the risk factors causing age-related skeletal muscle loss has not yet been developed, and it remains critical need to identify modifiable risk factors in order to guide the formulation of skeletal muscle loss prevention strategies. In this regard, chronic inflammation has been accepted as one of the accelerating factors causing skeletal muscle loss and sarcopenia (11–15).

Diet patterns have been shown to modulate inflammation and may therefore have different effects on skeletal muscle in view of their inflammatory potential (16–18). The inflammatory potential of diet patterns can be estimated using a validated tool, namely the Dietary inflammatory index (DII) (19). DII was derived from literature review of up to 2,000 research articles. It estimated the association of different food components (45 food components consisting of whole foods, nutrients as well as bioactive compounds) with six serum inflammatory cytokines [i.e., Interleukin (IL)-1β, IL-4, IL-6, IL-10, tumor necrosis factor (TNF)-α, and C-reactive protein (CRP)] (19). In general, a more pro-inflammatory diet corresponds to a higher DII score, while an anti-inflammatory diet corresponds to a lower DII score. However, the results of previous studies on the associations between DII and skeletal muscle strength, mass, and sarcopenia were inconsistent (20–25).

Meta-analysis as an effective means to synthesize the existing evidence may help fill this knowledge gap. Recently, a meta-analysis including 11 studies suggested that DII may be associated with sarcopenia (26). However, it did not conduct subgroup analyses based on diet and muscle mass assessment methods, which were two of the important sources of inter-study heterogeneity and bias for the association between DII and skeletal muscle. Besides, it only investigated sarcopenia, leaving the effect of DII on muscle strength and mass unclear. Therefore, a meta-analysis with more comprehensive included studies is necessary to further elucidate the association of DII with skeletal muscle strength, mass, and sarcopenia.

2. Methods

2.1. Protocol and registration

This study was reported according to the Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) (**Supplementary Appendix 1**) (27) and Meta-analyses Of Observational Studies in Epidemiology (MOOSE) guideline (**Supplementary Appendix 2**) (28). The protocol was prospectively registered in PROSPERO (CRD42022334333).

2.2. Search strategy

Three databases, namely Embase, Pubmed, and Web of Science, were searched from inception to March 12, 2023. The search strategy was constructed based on following keywords: ("DII" OR "dietary inflammatory index" OR "inflamma* AND diet") AND ("sarcopen*" OR "sarcopenia" OR "sarcopenic" OR "muscle mass" OR "muscle volume" OR "muscle quality" OR "muscle size" OR "lean mass" OR "grip strength" OR "hand strength" OR "muscle strength" OR "gripping strength" OR "holding power" OR "grip dynamometer" OR "handgrip" OR "muscular atrophy" OR "muscular dystrophy" OR "muscle dystrophy" OR "muscle atrophy"). A systematic search strategy was designed as broad as possible and adjusted according to databases (**Supplementary Appendix 3**). No language restriction was applied, and Google Translate was used for non-English articles (29). Reference manager software was applied to automatically remove duplicates. For finally included studies, the reference lists and related reviews were manually screened for additional studies meeting the eligibility criteria.

2.3. Eligibility criteria

The research question was specified using PICO (**Supplementary Appendix 4**). The inclusion criteria for studies were: (1) participants were adults aged 18 years or older; (2) intervention or exposure was diet patterns with different dietary inflammatory potential (evaluated by DII or energy-adjusted DII [E-DII]); (3) groups with high DII were compared with those with low DII; (4) outcomes included skeletal muscle strength, mass, and sarcopenia; (5) studies with observational study design (e.g., cross-sectional studies, case-control studies, and longitudinal studies).

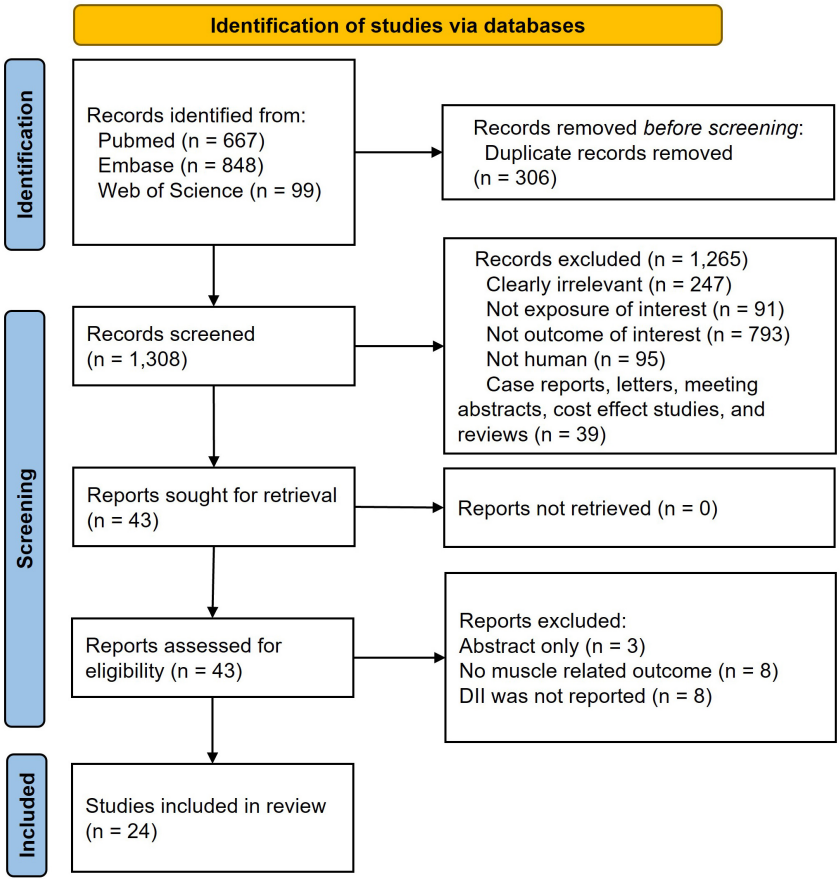

FIGURE 1
Preferred reporting items of systematic reviews and meta-analysis (PRISMA) flow chart.

2.4. Study selection and data extraction

After removal of duplicates, two investigators screened the titles and abstracts and then conducted full-texted assessment on the included studies independently. The agreement between two authors was acceptable for the titles and abstracts screening (Kappa statistic was 0.85), when disagreement was solved by discussion, and complete agreement was achieved in full-text assessment (Kappa statistic was 1.0). The desired data was extracted using a standardized table, which included study characteristics (i.e., author, year of publication, country, study type, and study setting), demographic information of participants [i.e., age, sex, and body mass index (BMI)], exposure measurements (DII reported as continuous variables, or category variables, for which the methods used for categorization were extracted as well), outcome measurements (muscle components reported as continuous variables, or category variables, and for which the methods used for categorization were extracted as well), effect sizes, and adjustments.

We extracted the adjusted odds ratio (OR) when comparing different DII category groups with the lowest group, or a β coefficient for the continuous association between DII and skeletal muscle. If a study reported results from diverse models with adjustments of different potential confounding factors, the most adjusted results would be chosen (30). We contacted the authors of studies with missing data for further information.

2.5. Quality assessment and certainty of evidence

The methodological quality was assessed by the Risk Of Bias In Non-randomized Studies (ROBINS-E) assessment tool. ROBINS-E was based on 7 domains including risk of confounding bias, selection bias, exposure measurement, departure from intended exposure bias, missing data, outcome measurement, and selection of reported bias (31). Articles were judged as low risk of bias if all criteria were low risk of bias, if at least one criterion was rated as moderate, serious, or critical risk of bias, the overall quality of study would be regarded as moderate, high, and very high risk of bias, respectively.

Two investigators assessed the methodological quality of each study independently. Any disagreement was resolved by discussion as far as possible; if failed, the corresponding author (Ning Wang) would be consulted to help make the final decision.

Certainty of evidence was assessed by using the Grades of Recommendation, Assessment, Development and Evaluation (GRADE) tool. Factors including within-study risk of bias, the indirectness of the evidence, heterogeneity, precision of the effect

TABLE 1 Characteristic of included studies.

Study	Country	Study type	Participants' health	Female (%)	Mean age (year)	BMI (kg/m^2)	Diet assessment	Comparison level	Outcome	Adjusted confounders
Bagheri et al. (24)	Iran	Cross-sectional	Without conditions causing sarcopenia other than age	50	66.7	27.4	FFQ	Top vs. bottom tertile	Low muscle strength/mass, sarcopenia (category)	Age, sex, energy intake, physical activity, smoking, alcohol consumption, medication use, and history of disease
Bian et al. (20)	China	Cross-sectional	Crohn's disease	27.86	32.6	20.7	FFQ	Top vs. bottom quartile	Low muscle strength/mass, sarcopenia (category)	Age, sex, BMI, smoking, alcohol consumption, nutritional status, Crohn's disease activity, and energy intake
Cervo et al. (43)	Canada	Cross-sectional	Community-dwelling older men without critical ill	0	81.1	27.7	Diet history questionnaire	Continuous	Muscle mass (continuous)	Age, smoking, calcium intake, physical activity, use of NSAIDs, use of bisphosphonates, presence of musculoskeletal disease, and comorbidity
Cervo et al. (42)	Australia	Cross-sectional	Community-dwelling older adults	51.14	63.0	27.9	FFQ	Continuous	Muscle strength/mass (continuous)	Age, percent body fat, smoking, steps per day, calcium, and alcohol intakes
Chen et al. (44)	USA	Cross-sectional	Community-dwelling adults	48.01	37.2	NA	24 h-dietary recall	Continuous/top vs. bottom tertile	Muscle strength/mass (continuous), and sarcopenia (category)	Age, sex, race, educational level, marriage, family poverty income ratio, smoking, drinking, physical activity, BMI, diabetes, and hypertension
Chen et al. (45)*	USA	Cross-sectional	Community-dwelling adults	51.0	62.1	27.7	24 h-dietary recall	Continuous/top vs. bottom tertile	Muscle strength/mass (continuous), low muscle mass (category), and sarcopenia (category)	Age, sex, race, education, marital status, nativity, smoking, physical activity, BMI, chronic disease, and protein
Davis et al. (46)	Australia	Longitudinal	Community-dwelling women	100	50.3	26.0	DQES	Continuous	Muscle mass (continuous)	Age, physical activity, smoking, protein, dietary energy
Davis et al. (47)*	Australia	Longitudinal	Community-dwelling adults	0	50.0	26.5	FFQ	Continuous	Muscle mass (continuous)	Age, fat mass, and physical activity
Esmaeily et al. (48)	Iran	Cross-sectional	Community-dwelling adults	66.0	77.0	29.0	FFQ	Continuous/top vs. bottom tertile	Muscle strength (continuous) and low muscle strength (category)	Age, family number, gender, CVD medication, BMI, and physical activity
Geng et al. (21)	USA	Cross-sectional	Community-dwelling adults	45.23	45.4	NA	24 h-dietary recall	Top vs. bottom tertile	Sarcopenia (category)	Age, gender, race, ratio of family income to poverty, education level, marital, BMI, comorbidity, smoking, alcohol, physical activity
Gojanovic et al. (49)	Australia	Cross-sectional	Community-dwelling adults	34.36	66.4	27.7	DQES	Continuous	Muscle mass (continuous)	Age, sex, and body fat percentage
Haß et al. (50)	German	Cross-sectional	Healthy old adults	75	72.4	28.8	24 h-dietary recall	Continuous	Muscle strength/mass (continuous)	Age, sex, and physical activity
Huang et al. (22)	USA	Cross-sectional	Chronic kidney disease patients	54.89	55.6	NA	24 h-dietary recall	Continuous/top vs. bottom tertile	Muscle mass (continuous) and sarcopenia (category)	Age, gender, race, income, physical activity, smoking, alcohol, diabetes, hypertension, overweight, central obesity, comorbidity, eGFR, ACR, hypoalbuminemia, low energy intake, low protein intake, CRP, WBC, NLR, and NHANES strata

(Continued)

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TABLE 1 (Continued)

Study	Country	Study type	Participants' health	Female (%)	Mean age (year)	BMI (kg/m^2)	Diet assessment	Comparison level	Outcome	Adjusted confounders
Inoue et al. (51)	Japan	Cross-sectional	Ambulatory patients aged 65 years or older without obvious disability due to certain disease	77.6	67.4	24.1	BDHQ	Top vs. bottom quartile	sarcopenia, low muscle strength and mass (category)	Age, sex, comorbidity, physical activity, BMI, protein intake, and energy intake
Jin et al. (52)	Korea	Cross-sectional	Community-dwelling menopause women	100	63.5	NA	3-days food record	Continuous	Muscle strength/mass (continuous)	Age, BMI, menopausal period, smoking, alcohol, vitamin D supplement intake, and physical activity.
Kim and Park (53)	Korea	Cross-sectional	Community-dwelling adults	61.1	76.9	24.6	24 h-dietary recall	Not reported	Low muscle strength (category)	Age, chewing ability, and energy intake
Laclaustra et al. (54)	Spain	Longitudinal	Community-dwelling adults	51.5	68.4	28.5	Computer-based diet history	Top vs. bottom tertile	Low muscle strength (category)	Age, sex, education, smoking, BMI, energy intake, comorbidity, time spent watching TV, and physical activity
Linton et al. (58)	Australia	Cross-sectional	Functionally able, community-dwelling adults	76.4	72.1	25.8	24 h-dietary recall	Continuous	Muscle strength/mass (continuous)	Age, gender, waist circumference, comorbidity, and physical activity
Park et al. (23)	USA	Cross-sectional	Community-dwelling women	100	62.3	25.7	24 h-dietary recall	Top vs. bottom halves	Sarcopenia (category)	Age, family income, regular exercise, education, smoking and female hormone supplements
Son et al. (25)	Japan	Cross-sectional	Community-dwelling adults	48.2	74.6	22.2	BDHQ	Top vs. bottom tertile	Low muscle strength/mass (category) and sarcopenia (category)	Age, education, protein intake, physical activity, comorbidity, eating alone, Lubben Social Network Scale (LSNS) social ties (<12), Geriatric Depression Scale (GDS) ≥ 6, and Geriatric Oral Health Assessment Index (GOHAI) score
Song et al. (55)	Korea	Cross-sectional	Community-dwelling women	100	57.7	24.3	3-days food record	Top vs. bottom tertile	Muscle mass (continuous)	Age, menopausal period, smoking, alcohol, BMI, and physical activity
Su et al. (56)	China	Longitudinal	Community-dwelling adults	50	72.5	23.7	FFQ	Top vs. bottom tertile	Sarcopenia (category)	Age, BMI, smoking, comorbidity, vitamin D status, and physical activity
Su et al. (57)*	China	Cross-sectional	Community-dwelling adults	52.5	71.9	23.7	FFQ	Continuous	Muscle strength/mass (continuous)	Age, corresponding measurement, BMI, smoking, physical activity, previous fracture, hypertension, diabetes, chronic obstructive lung disease, cardiovascular disease, rheumatoid arthritis, non-steroidal anti-inflammatory agent use, and osteoporosis medication
Xie et al. (59)	USA	Cross-sectional	Community-dwelling adults	51.0	51.7	29.2	24 h-dietary recall	Continuous	Muscle strength (continuous)	Age, gender, race, education, marital status, physical activity, energy intake, smoking

FFQ, Food Frequency Questionnaires; DQES, Dietary Questionnaire for Epidemiological Studies; BDHQ, brief self-administered diet history questionnaire; DII, Dietary inflammatory index; E-DII, energy-adjusted DII; SMI, skeletal muscle index; TUG, Up-ana-Go test; NA, not available. Symbol * was used to identify different articles with the same author surnames and publication time.

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or association estimates, and publication bias were considered to reach an overall certainty of the evidence rating of very low, low, moderate, or high for each outcome (32).

2.6. Statistical analysis

We grouped studies according to the methods used for reporting DII exposure and skeletal muscle outcomes. Three main methods of reporting results were used: (1) β coefficient for the continuous association between DII exposure and skeletal muscle strength or mass; (2) ORs for the risk of low muscle strength or mass comparing participants having the highest DII with those having the lowest DII; and (3) ORs for the risk of sarcopenia comparing participants having the highest DII with those having the lowest DII.

Random-effects model assumes that different studies estimate different but related effects, and yields identical results as the fixed-effects model in the absence of heterogeneity. Therefore, to obtain conclusions generalized to wilder arrays of situations, we used random-effects model in all analyses (33–35). The statistical heterogeneity of the included studies was examined by Cochrane's Q test and I^2 ($P < 0.05$ indicated statistically significant heterogeneity and $I^2 > 50\%$ indicated high-degree heterogeneity (36).

For studies reporting relative risks of sarcopenia for several categories with number of cases and controls, we further conducted a dose-response analysis (37). The median or mean of DII for each category was assigned to each corresponding OR. The dose-response meta-analysis was conducted using restricted cubic spline models with 3 knots to estimate potential non-linear trend in each study, and the results from included studies were pooled using random-effect multivariate meta-analysis (38).

Since the predictive ability of DII scores based on 27–45 food parameters were validated in previous studies (22, 39), sensitivity analyses omitting studies that calculated DII scores based on less than 27 components were performed. Additional sensitivity analyses were performed by removing each single study from the analysis to assess the robustness of the findings. Subgroup studies were carried out according to geographic region (different continents), age (<65 and ≥65 years old), BMI [overweight/obese (BMI ≥ 25kg/m^2) and normal/underweight (BMI < 25kg/m^2)], diet assessment methods, muscle mass assessment methods, and the definition of sarcopenia [European Working Group on Sarcopenia in Older People (EWGSOP), Asian Working Group for Sarcopenia (AWGS), Foundation for The National Institute of Health (FNIH), and low appendicular skeletal muscle mass (ASM)]. Since several studies did not present the mean BMI of participants, they were precluded in the subgroup analyses for BMI. The risk of publication bias was analyzed by funnel plots with Egger test, using Duval and Tweedie trim-and-fill method for adjustment of funnel plot asymmetry (40, 41). *P* values less than 0.05 were considered statistically significant. Comprehensive Meta-Analysis (CMA) software V.3.3.0 (Biostat) was used for meta-analysis and Stata V.14 was used for dose-response meta-analysis and publication bias assessment.

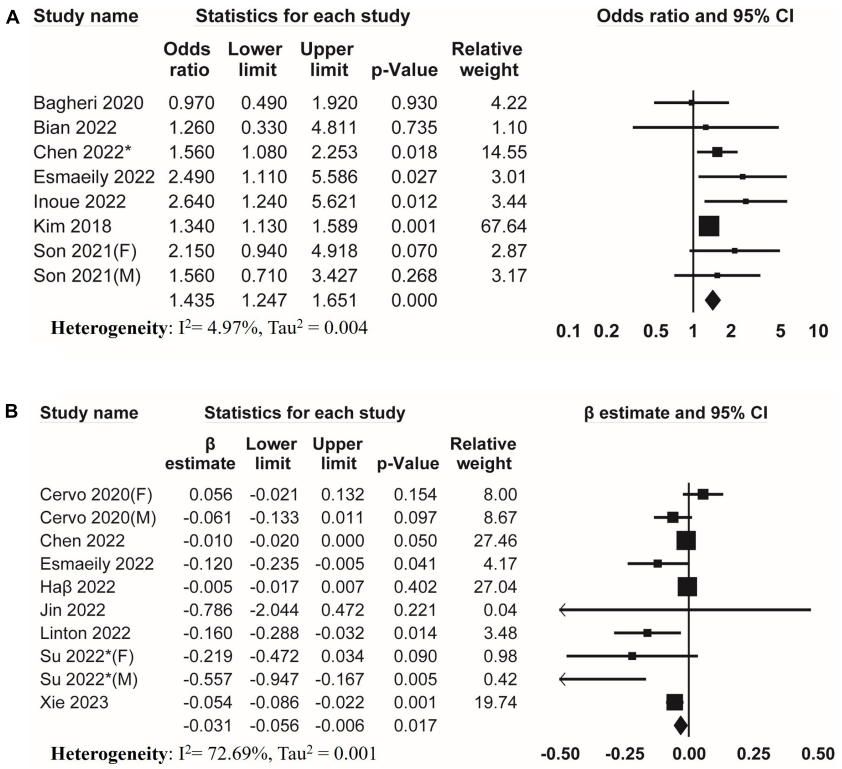

FIGURE 2
Forest plots of meta-analyses on the association between DII and muscle strength. **(A)** Forest plot of meta-analysis on the association between DII and risk of low muscle strength; **(B)** forest plot of meta-analysis on the association between DII and muscle strength.

3. Results

3.1. Study selection and characteristics

Of the 1,308 records identified by initial search after removal of duplicates, 1,265 obviously ineligible studies were excluded in the titles and abstracts screen, resulting in 43 articles for full-text assessment. Eventually, 24 researches involving 56,536 participants met the eligibility criteria (**Figure 1**). Twenty studies were cross-sectional designed and four were longitudinal studies (20–25, 42–59). No interventional study was available for this meta-analysis.

Table 1 and **Supplementary Appendix 5** summarized the detailed characteristics of the included studies. The number of participants ranged from 79 to 25,781, with the mean age ranging from 32.6 to 81.1 years. The mean BMI was higher than 25 kg/m^2 in 13 studies (54.2%) and lower than 25 kg/m^2 in 7 studies (29.2%), and the remaining 4 studies (16.7%) did not report a mean BMI. The largest proportion of studies (41.7%) were carried out in Asia, followed by North America (29.2%). The choice of measurement of skeletal muscle mass was also different, with DXA (80%) being the most widely used, followed by bio-impedance analysis (BIA) (20%). The handgrip strength was the most frequent choice of measurement of skeletal muscle strength. Low muscle strength and mass were defined either by recommended cut-off values for the participants or based on the population-specific thresholds. The diagnosis of sarcopenia varied across five different diagnostic criteria, including EWGSOP 1, EWSOP 2, FNIH, AWGS 2019, and a criterion based on low ASM alone. Among them, AWGS 2019 (35.7%) was mostly used. The ROBINS-E tool suggested moderate to high risk of bias for most studies, and very high risk of bias for one study. In most included studies, bias originated from uncontrolled confounding bias and missing data (**Supplementary Appendix 6**).

3.2. Association between DII and skeletal muscle strength

Seven studies investigated the association between DII and low skeletal muscle strength. The result of meta-analysis revealed a positive association between DII and low skeletal muscle strength (pooled OR = 1.435, 95% CI, 1.247–1.651) (**Figure 2A**) without evidence of substantial heterogeneity (I^2 = 4.97%, Tau^2 = 0.004). Eight studies reported β from multiple linear regression. The result of meta-analysis of these studies showed a negative association of DII with skeletal muscle strength (pooled β = −0.031, 95% CI, −0.056 to −0.006, I^2 = 72.69%, Tau^2 = 0.001) (**Figure 2B**).

3.3. Association between DII and skeletal muscle mass

Regarding skeletal muscle mass, six studies investigated the association between DII and low skeletal muscle mass (**Figure 3A**). The result of meta-analysis suggested that DII was associated with low skeletal muscle mass (pooled OR = 1.106, 95% CI, 1.058–1.157) without evidence of heterogeneity (I^2 = 0%, Tau^2 = 0). Moreover, meta-analysis of eleven studies suggested that DII was negatively associated with muscle mass (pooled β = −0.099, 95% CI, −0.145 to −0.053) with significant heterogeneity (I^2 = 88.67%, Tau^2 = 0.005) (**Figure 3B**).

3.4. Association between DII and sarcopenia

Nine studies examined the association between DII and sarcopenia. Meta-analysis of these studies covering 41,233 participants revealed that higher DII was associated with an increased risk of sarcopenia (pooled OR = 1.530, 95% CI, 1.245–1.880) (**Figure 4**). An I^2 of 69.46% with Tau^2 of 0.045 indicated significant heterogeneity among studies.

For dose-response meta-analysis, we included seven studies that divided DII into at least three categories, and two studies were excluded (23, 44). Setting the reference DII level as −2.68, we found a significant non-linear dose-response relationship between DII and sarcopenia (P = 0.012 for non-linearity). No increased risk was observed with higher DII when DII was lower than 0. The risk of sarcopenia increased significantly with DII when DII was at the range of 1–4.315 (**Figure 5**).

3.5. Sensitivity analyses

Sensitivity analyses omitting studies that calculated DII scores based on less than 27 food parameters altered the results on low muscle mass without significant impact on the heterogeneity (**Table 2**). In addition, omitting each single study did not alter the findings (**Supplementary Appendix 7**), suggesting that the results were robust.

3.6. Subgroup analyses

We further conducted subgroup analyses by stratifying studies according to geographic regions, age, BMI, diet assessment methods, muscle mass assessment methods, and sarcopenia diagnostic criteria (**Table 3**), and the results were reported according to outcomes as follows:

Low muscle strength: Geographic regions and age had no impact on the association between DII and low muscle strength, and no significant heterogeneity was observed among these subgroups.

Muscle strength: In subgroup analyses, subgroups for Asia (pooled β = −0.246, 95% CI −0.446 to −0.045, studies = 3, I^2 = 46.13%, Tau^2 = 0.02), normal/underweight (pooled β = −0.244, 95% CI −0.426 to −0.062, studies = 2, I^2 = 44.65%, Tau^2 = 0.01), and Food Frequency Questionnaires (FFQ) (pooled β = −0.1.07, 95% CI −0.209 to −0.005, studies = 4, I^2 = 74.82%, Tau^2 = 0.01) revealed a significant association between DII and muscle strength without substantial heterogeneity. We failed to downgrade the heterogeneity in subgroup analyses.

Low muscle mass: The significant association between DII and low skeletal muscle mass was altered in subgroup analyses; we

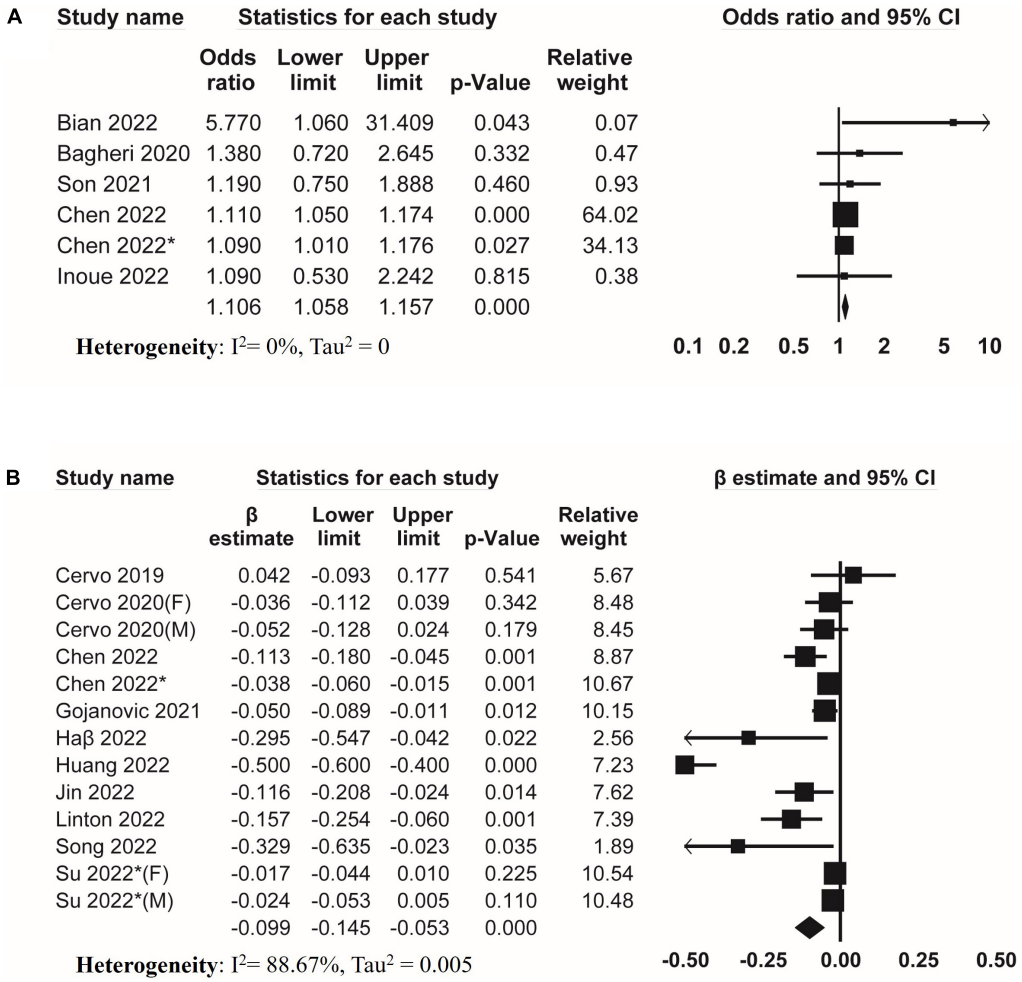

FIGURE 3
Forest plots of meta-analyses on the association between DII and muscle mass. **(A)** Forest plot of meta-analysis on the association between DII and risk of low muscle mass; **(B)** forest plot of meta-analysis on the association between DII and muscle mass.

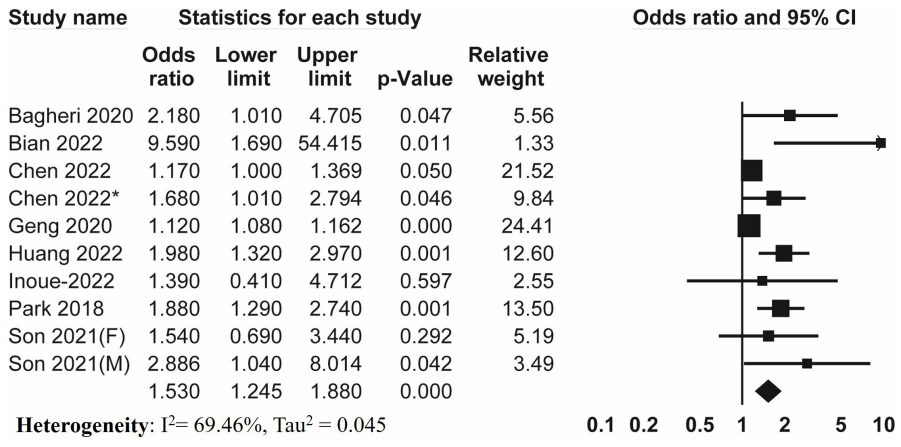

FIGURE 4
Forest plots of meta-analyses on the association between DII and sarcopenia.

found a significant association between DII and low skeletal muscle mass in subgroups for North America (pooled OR = 1.103, 95% CI 1.055–1.154, studies = 2, I^2 = 0%, Tau^2 = 0), participants younger than 65 years old (pooled OR = 1.105, 95% CI 1.022–1.195, studies = 3, I^2 = 47.42%, Tau^2 = 0), overweight/obese (pooled OR = 1.094, 95% CI 1.014–1.180, studies = 2, I^2 = 0%, Tau^2 = 0),

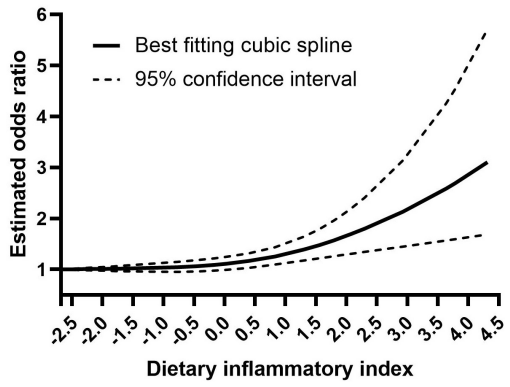

FIGURE 5
Dose-response analysis of the association between DII and sarcopenia.

24h-dietary recall (pooled OR = 1.104, 95% CI 1.056–1.154, studies = 3, I^2 = 0%, Tau^2 = 0), and DXA (pooled OR = 1.104, 95% CI 1.056–1.155, studies = 4, I^2 = 0%, Tau^2 = 0) without substantial heterogeneity.

Muscle mass: In the primary analysis, we observed substantial inconsistency between included studies, but we failed to downgrade the heterogeneity in all subgroups except for the subgroup only including the overweight/obesity population (pooled β = −0.042, 95% CI −0.065 to −0.019 studies = 5, I^2 = 12.54%, Tau^2 = 0) and subgroup using BIA for muscle mass assessment (pooled β = −0.309, 95% CI −0.503 to −0.114 studies = 2, I^2 = 0%, Tau^2 = 0).

Sarcopenia: Most subgroup analyses did not alter the significant association between DII and sarcopenia except for the subgroup for the groups that used FFQ to assess diet. Only in the subgroup involving older participants (≥65 years) and subgroup of overweight/obese, heterogeneity was reduced, suggesting that age might account for the heterogeneity to a certain extent.

3.7. Publication bias

The funnel plots showed asymmetry among outcomes of muscle strength, mass, and sarcopenia (**Supplementary Appendix 8**) with results of Egger test suggesting evidence of publication bias (**Table 4**). The adjusted effect estimates showed similar results with primary analyses in outcomes of low muscle strength, low muscle mass while the trim-and-fill analysis alter the significant association of DII with muscle strength and sarcopenia; no adjustment was needed for the analysis of muscle mass (**Supplementary Appendix 8** and **Table 4**).

However, these results should be interpreted with caution since Egger test is not accurate when the number of included studies is small (40, 41).

3.8. Certainty of evidence

We assessed the certainty of evidence using GRADE. The association between DII and low muscle mass was of low certainty, and the associations of DII with low muscle strength, muscle strength, muscle mass and sarcopenia were of very low certainty (**Table 5**).

4. Discussion

This meta-analysis explored the associations of DII with skeletal muscle strength, mass, and sarcopenia, and the results showed that DII was correlated with both low skeletal muscle strength and mass. Consistently, a higher DII was associated with an increased risk of sarcopenia. Our dose-response meta-analysis showed that the risk of sarcopenia was at the lowest point when DII was −2.68 to 0, and increased DII raised the risk of sarcopenia when DII was higher than 0.

4.1. Comparison with previous studies

An earlier systematic review and meta-analysis reported the association between adherence to a Mediterranean diet and physical performance in older adults (18), while another systematic review by Bloom et al. suggested that a healthier diet was associated with a decreased risk of sarcopenia in the aged people (17). Both studies indicated that diet habits might influence the skeletal muscle condition in older adults. The dietary inflammatory potential has been demonstrated to influence health outcomes as one of the modulators for systematic inflammation (19, 60, 61). Recently, a meta-analysis including 11 studies suggested that DII may be associated with sarcopenia (26). Yet, it did not conduct subgroup

TABLE 2 Sensitivity analyses omitting studies using less than 27 components in DII calculation.

Outcome	Number	Pooled OR (95% CI)	Pooled β (95% CI)	*P*-value for estimated effect	I^2 (%)	Tau^2
Low muscle strength	3	1.314 (1.115, 1.548)	–	**0.001**	0.00	0.00
Muscle strength	5	–	−0.074 (−0.136, −0.012)	**0.019**	78.22	0.00
Low muscle mass	3	1.339 (0.819, 2.186)	–	0.244	50.64	0.10
Muscle mass	6	–	−0.156 (−0.247, −0.066)	**0.001**	93.97	0.01
Sarcopenia	6	1.475 (1.169, 1.861)	–	**0.001**	78.59	0.05

OR, odds ratio. Values in bold indicated statistically significant results.

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TABLE 3 Subgroup analyses.

Outcome and subgroup	Classification	No	Pooled results of subgroups		Heterogeneity of subgroups	
			OR/β (95% CI)	*P*-value	I^2(%)	Tau^2
Low muscle strength						
Continents	Asia	6	1.507 (1.191,1.907)	**0.001**	15.88	0.02
	North America	1	1.560 (1.080, 2.253)	**0.018**	–	–
Age	≥65 years	5	1.565(1.182, 2.071)	**0.002**	29.61	0.04
	<65 years	2	1.537 (1.078, 2.191)	**0.018**	0	0.00
BMI	Overweight/Obesity	3	1.515 (0.994, 2.310)	0.053	35.80	0.05
	Normal/underweight	4	1.428 (1.198, 1.702)	<**0.001**	1.89	0.00
Diet assessment method	24 h-dietary recall	3	1.403 (1.208, 1.629)	<**0.001**	0.00	0.00
	FFQ	3	1.443 (0.765, 2.720)	0.257	35.14	0.11
	BDHQ	1	2.640 (1.240, 5.621)	**0.012**	–	–
Muscle strength						
Continents	Asia	3	−0.246 (−0.446, −0.045)	**0.016**	46.13	0.02
	Australia	2	−0.046 (−0.158, 0.065)	0.415	78.92	0.01
	Europe	1	−0.005 (−0.017, 0.007)	0.402	–	–
	North America	2	−0.029 (−0.072, 0.014)	0.180	84.89	0.00
Age	≥65 years	3	−1.41 (−0.291, 0.010)	0.067	78.80	0.02
	<65 years	5	−0.023 (−0.060, 0.015)	0.243	68.94	0.00
BMI	Overweight/Obesity	6	−0.019 (−0.040, 0.001)	0.058	65.99	0.00
	Normal/underweight	2	−0.244 (−0.426, −0.062)	**0.009**	44.65	0.01
Diet assessment method	24 h-dietary recall	3	−0.016 (−0.033, 0.001)	0.068	74.86	0.00
	FFQ	4	−0.107 (−0.209, −0.005)	**0.039**	74.82	0.01
	3-days food record	1	−0.786 (−2.044, 0.472)	0.221	–	–
Low muscle mass						
Continents	Asia	4	1.301 (0.909, 1.864)	0.151	11.00	0.02
	North America	2	1.103 (1.055, 1.154)	<**0.001**	0.00	0.00
Age	≥ 65 years	3	1.214 (0.870, 1.695)	0.254	0.00	0.00
	<65 years	3	1.105 (1.022, 1.195)	<**0.013**	47.42	0.00
BMI	Overweight/Obesity	2	1.094 (1.014, 1.180)	**0.021**	0.00	0.00
	Normal/underweight	3	1.353 (0.761, 2.408)	0.303	39.61	0.11
Diet assessment method	24 h-dietary recall	3	1.104 (1.056, 1.154)	<**0.001**	0.00	0.00
	FFQ	2	2.258 (0.896, 8.559)	0.231	58.10	0.60
	BDHQ	1	1.090 (0.530 2.242)	0.815	–	–
Muscle mass assessment method	DXA	4	1.104 (1.056, 1.155)	<**0.001**	0.00	0.00
	BIA	2	2.105 (0.476, 9.305)	0.326	67.79	0.85
Muscle mass						
Continents	Asia	3	−0.040 (−0.082, 0.002)	0.061	61.88	0.00
	Australia	3	−0.063 (−0.104, −0.022)	**0.003**	33.49	0.00
	Europe	1	−0.295 (−0.547, −0.042)	**0.022**	–	–
	North America	4	−0.152 (−0.331, 0.027)	0.095	96.36	0.03
Age	≥65 years	5	−0.043 (−0.080, −0.006)	**0.021**	64.14	0.00
	<65 years	6	−0.150 (−0.251, −0.049)	<**0.004**	92.93	0.02
BMI	Overweight/Obesity	5	−0.042 (−0.065, −0.019)	<**0.001**	12.54	0.00
	Normal/underweight	3	−0.027 (−0.047, −0.008)	**0.007**	73.25	0.00

(Continued)

TABLE 3 (Continued)

Outcome and subgroup	Classification	No	Pooled results of subgroups		Heterogeneity of subgroups	
			OR/β (95% CI)	*P*-value	I^2(%)	Tau^2
Diet assessment method	24 h-dietary recall	4	−0.227 (−0.421, −0.033)	**0.022**	96.40	0.04
	FFQ	3	−0.038 (−0.069, −0.007)	**0.017**	49.58	0.00
	3-days food record	1	−0.116 (−0.208, −0.024)	**0.014**	–	–
	BDHQ	1	−0.329 (−0.635, −0.023)	**0.035**	–	–
	Diet history questionnaire	1	−0.042 (−0.093, 0.177)	0.541	–	–
Muscle mass assessment method	DXA	9	−0.089 (−0.135, −0.042)	<**0.001**	89.89	0.01
	BIA	2	−0.309 (−0.503, −0.114)	**0.002**	0.00	0.00
Sarcopenia						
Continents	Asia	4	2.164 (1.364, 3.431)	**0.001**	7.46	0.02
	North America	5	1.386 (1.135, 1.692)	**0.001**	76.56	0.03
Age	≥65 years	3	1.939 (1.232, 3.051)	**0.004**	0.00	0.00
	<65 years	6	1.452 (1.164, 1.812)	**0.001**	78.14	0.04
BMI	Overweight/Obesity	3	1.853 (1.398, 2.456)	<**0.001**	0.00	0.00
	Normal/underweight	3	2.260 (1.158, 4.411)	**0.017**	30.56	0.14
Diagnostic criteria	EWGSOP	1	2.180 (1.010, 4.705)	**0.047**	–	–
	Low muscle mass	1	1.880 (1.290, 2.740)	**0.001**	–	–
	AWGS 2019	3	2.260 (1.158, 4.411)	**0.017**	30.56	0.39
	FNIH	4	1.279 (1.067, 1.532)	**0.008**	70.30	0.02
Diet assessment method	24 h-dietary recall	6	1.434 (1.178, 1.746)	<**0.001**	71.19	0.03
	FFQ	2	3.696 (0.920, 14.847)	0.065	57.23	0.63
	BDHQ	1	1.390 (0.410, 4.712)	0.597	—	—
Muscle mass assessment method	DXA	7	1.421 (1.171, 1.725)	<**0.001**	69.90	0.03
	BIA	2	2.730 (1.137, 6.553)	**0.025**	46.50	0.28

No, numbers of studies; OR, odds ratio. Values in bold indicated statistically significant results.

analyses based on diet and muscle mass assessment methods, which were two of the important sources of inter-study heterogeneity and bias for the association between DII and skeletal muscle. For example, 24-h recall was less biased than FFQ while FFQ worked better on episodically consumed nutrient and food (62). Moreover, it only investigated sarcopenia, leaving the effect of DII on muscle strength and mass unclear. Given that muscle strength and mass decline at different speeds and independently predispose old adults to risk of adverse events (63), assessing the impact of DII on muscle strength and mass separately is favorable. In response to this situation, our research summarized all the available studies, took these potential confounders into consideration, and provided more comprehensive evidence for the effect of DII on skeletal muscle.

4.2. Possible explanations

The DII was formulated based on extensive literature including evidences from a wide range of human populations with different study designs and dietary measurements, and also evidence from animal and cell experiments (64). An advantage of DII is that it takes the whole diet into account, not just individual nutrients or foods (19). Previous studies have substantiated the utility of

TABLE 4 Risk of publication bias of included studies in meta-analysis based on Egger test and results of trim-and-filled analysis.

Outcome	Egger test		Trim-and-fill analysis	
	t-Value	*P*-value	Studies trimmed	Adjusted estimates
Low muscle strength	1.46	0.194	3	**1.360 (1.096, 1.687)**
Muscle strength	3.05	**0.016**	4	−0.022 (−0.051, 0.006)
Low muscle mass	1.86	0.136	2	**1.103 (1.029, 1.183)**
Muscle mass	2.35	**0.039**	0	**−0.099 (−0.145, −0.053)**
Sarcopenia	5.38	**0.001**	6	1.149 (0.943, 1.400)

Values in bold indicated statistically significant results.

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TABLE 5 Summary of findings (Sof) by GRADE system.

Outcome	Studies number	Risk of bias	Inconsistency	Indirectness	Imprecision	Publication bias	Other consideration	Effect size (95% CI)	Certainty[a]
Low muscle strength	7	Serious	Not serious	Not serious	Not serious	Not serious	None	OR: 1.435 (1.247, 1.651)	⊕◯◯◯ Very low due to risk of bias
Muscle strength	8	Serious	Serious	Not serious	Not serious	Serious	None	β: −0.031 (−0.056, −0.006)	⊕◯◯◯ Very low due to risk of bias, inconsistency, and publication bias
Low muscle mass	6	Not serious	Not serious	Not serious	Not serious	Not serious	None	OR: 1.106 (1.058, 1.157)	⊕⊕◯◯ Low
Muscle mass	11	Serious	Serious	Not serious	Not serious	Serious	None	β: −0.099 (−0.145, −0.053)	⊕◯◯◯ Very low due to risk of bias, inconsistency, and publication bias
Sarcopenia	9	Serious	Serious	Not serious	Not serious	Serious	Upgraded for dose-response relationship	OR: 1.530 (1.245, 1.880)	⊕◯◯◯ Very low due to risk of bias, inconsistency, and publication bias

OR, odds ratio.

[a]High certainty: We are very confident that the true effect lies close to that of the estimate of the effect; Moderate certainty: We are moderately confident in the effect estimate. The true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different; Low certainty: Our confidence in the effect estimate is limited. The true effect may be substantially different from the estimate of the effect; Very low quality: We have very little confidence in the effect estimate: The true effect is likely to be substantially different from the estimate of effect.

DII as a tool to characterize the inflammatory potential of diets and to predict the risk of multiple health conditions including colorectal cancer, cardiovascular diseases, and depression (65–68). Furthermore, DII was also used in epidemiologic studies to assess the potential association between diet and skeletal muscle aging.

All the included studies had observational design in nature, which were susceptible to confounding factors. Our findings were independent of certain confounding factors since most of the studies involved in meta-analysis adjusted their results for age, gender, and physical activity. However, residual confounding by some unmeasured factors and other unknown factors cannot be ruled out. For instance, the majority of included studies reported incomplete adjustment for some important confounders such as energy intake and comorbidity.

How diet associates with skeletal muscle aging can be partly explained by the systemic chronic inflammation that may lead to anabolic resistance and muscle stem cells (MuSCs) dysfunction (69–71). A systematic review and meta-analysis found that systemic inflammatory cytokines [including, CRP, IL-6, and tumor necrosis factor α (TNFα)] were negatively associated with muscle strength and muscle mass (15). Specifically, the dysregulated systemic chronic inflammation activates the ubiquitin-proteasome system by inhibiting the activity of insulin-like growth factor 1 (IGF-1) (72), leading to anabolic resistance and loss of muscle homeostasis in the aged people (69, 73). In chronic systemic inflammation, an increase in both M1 pro- and M2 anti-inflammatory macrophages was observed (74). The increased M1 macrophages account for higher levels of pro-inflammation cytokines (e.g., IL-1β, TNF-α, Interferon-γ). These cytokines will result in muscle dystrophies by impairing the regenerative function of resident MuSCs (70, 71). M2 macrophages can induce extracellular matrix accumulation and muscle fibrosis and impair the function of MuSCs, so as to affect skeletal muscle regeneration (75, 76). Given the information mentioned above, it is not surprising to find a positive association between pro-inflammatory diet (high DII) and skeletal muscle aging.

4.3. Strengths and limitations

In this study, we performed a systematic literature search across several bibliographic databases and included 24 observational studies in our meta-analysis. To the best of our knowledge, our work provided up-to-date finding on the associations between DII and skeletal muscle aging. More specifically, by taking into account muscle strength, muscle mass, and sarcopenia, we delivered an overview of evidence regarding how DII was related to skeletal muscle decline in adults. However, our work was also subjected to several limitations. Firstly, many of the included studies were cross-sectional designed, so causal conclusions could not be established based on our analysis results. Therefore, the findings require further validation by longitudinal or interventional studies. Secondly, the findings should be interpreted cautiously since evidence of publication bias was identified in the results of muscle strength, muscle mass, and sarcopenia. Nevertheless, the publication bias may be unreliable due to the small number of included studies in some outcomes (i.e., low muscle strength, muscle strength, and low muscle mass), and this may be changed

with the increase of evidence in the future. Thirdly, several studies estimated muscle mass using BIA. Although BIA was validated as comparable to DXA (77), our meta-analysis may suffer from different equations that were used to estimate muscle mass. Finally, substantial heterogeneity was observed in certain groups. Our subgroup analyses suggested that region, age, and BMI were important sources of heterogeneity. However, residual heterogeneity was still observed. Previous studies implied that the number of dietary components used for DII calculation and the definition of sarcopenia might introduce significant inter-study heterogeneity, but the insufficiency of studies limited the power of such subgroup analyses (22, 39, 78). Therefore, more evidence with consistent methods for DII assessment and sarcopenia diagnosis is required to improve analysis and identify the sources of heterogeneity.

4.4. Clinical and research implications

Numbers of factors are responsible for malnutrition in older adults (79, 80), and malnutrition is a major risk factor for the age-related skeletal muscle decline (81). Sufficient nutrition plays a fundamental role in preserving skeletal muscle strength, mass, and function in older adults (82). Some evidence suggested that healthier diet patterns with adequate consumption of proteins, antioxidant nutrients, and long-chain polyunsaturated fatty acids exerted a positive effect on the prevention of skeletal muscle loss (82). However, the relationship between the dietary inflammatory potential and skeletal muscle was less clear.

In this study, a positive association between pro-inflammatory diet (high DII) and loss of skeletal muscle was observed. Based on this, a diet strategy with increased intake of anti-inflammatory dietary components (e.g., vegetables and fruits) and decreased intake of pro-inflammatory components (e.g., sugar-sweetened drinks and processed meat) is expected to be preventive for skeletal muscle health. Our finding suggested that the DII should be cautiously considered in formulating nutritional intervention recommendations for older adults from the aspect of skeletal muscle loss management. We also implied the utility of DII as a tool to predict the risk of skeletal muscle loss. Moreover, our results reinforced the public awareness of the pro-inflammatory property of diet and the need to avoid exposure to the risk of inflammation, and highlighted the rationale for DII control for the purpose of preventing skeletal muscle loss in older adults.

5. Conclusion

In summary, our meta-analysis suggested that higher dietary inflammatory potential was significantly associated with lower skeletal muscle strength, mass, and higher prevalence of sarcopenia. A larger number of longitudinal or interventional studies with consistent assessment and standardized methodology are needed to further explore the association between dietary inflammatory potential and skeletal muscle in the future.

Data availability statement

The original contributions presented in this study are included in the article/**Supplementary material**, further inquiries can be directed to the corresponding author.

Author contributions

NW and HX conceived the study. HW and HX were responsible for the design of the study and study selection and did the data extraction and risk of bias assessment. YL, WL, and ZW contributed to preparation and data analysis. NW contributed to the revision of the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

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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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2023.1100918/full#supplementary-material

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Sarcobesity, but not visceral fat, is an independent risk factor for complications after radical resection of colorectal cancer

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Background: The influence of body composition on the outcome of colorectal cancer surgery is controversial. The aim of this study was to evaluate the effects of visceral obesity and sarcobesity on the incidence of total and surgical complications after radical resection of colorectal cancer.

Methods: We collected a total of 426 patients who underwent elective radical resection of colorectal cancer at Beijing Friendship Hospital, Capital Medical University from January 2017 to May 2018. According to the inclusion and exclusion criteria, 387 patients were finally included. A CT scan at the level of the L3-L4 intervertebral disk was selected to measure the values of visceral fat area and skeletal muscle area. Multivariate analysis was used to explore the independent risk/protective factors affecting postoperative complications.

Results: 128 (33.1%) patients developed complications, and 44 (11.4%) patients developed major complications. Among them, 111 patients developed surgical complications and 21 developed medical complications. Visceral fat area (Z =−3.271, p =0.001), total fat area (Z=−2.613, p =0.009), visceral fat area to subcutaneous fat area ratio (V/S, Z=−2.633, p =0.008), and sarcobesity index (Z =−2.282, p =0.023) were significantly associated with total complications. Visceral fat area (Z =−2.119, p =0.034) and V/S (Z =−2.010, p =0.044) were significantly associated with total surgical complications. Sarcobesity index, smoking, stoma, blood loss, surgery time, and American Society of Anesthesiology (ASA) score were selected as risk factors for total postoperative complications according to LASSO regression. Multivariate logistic regression analysis suggested that sarcobesity index was an independent risk factor for postoperative total complications and surgical complications. Subgroup analysis suggested that albumin level was an independent protective factor for postoperative total complications in male patients. Smoking, operative time, and sarcobesity index were independent risk factors, and cholesterol was an independent protective factor for total postoperative complications in female patients.

Conclusion: Increased sarcobesity index is an independent risk factor for postoperative complications in patients with colorectal cancer, while visceral fat area is not. For female patients, smoking, operation time, and obesity index are independent risk factors for postoperative complications, while cholesterol is an independent protective factor. For male patients, serum albumin is an independent protective factor for postoperative complications.

KEYWORDS

colorectal cancer, sarcobesity, sarcobesity index, visceral fat area, postoperative complications

Introduction

Overweight and obesity are important global health issues with an important role in the development and prognosis of several cancers (1). Obesity increases the difficulty of surgery, incurs high costs, and complicates the surgical treatment of colorectal cancer (2, 3). Body mass index (BMI) is commonly used to determine obesity. However, BMI as a risk profiler for early postoperative outcomes has been questioned (4–6). The association between obesity as diagnosed by BMI and adverse outcomes after colorectal cancer surgery remains controversial (7, 8). These conflicting data may be attributed to the inability of BMI to assess the proportions of fat and lean tissue (9).

The distribution of adipose tissue in the body is diverse, and different fat depots have different metabolic activities. Visceral fat is considered a more accurate parameter than subcutaneous fat to reflect dysfunctional adipose tissue, which is a major cause of various obesity-related comorbidities (10). Visceral fat is metabolically active, which can lead to a chronic inflammatory state and increase the risk of insulin resistance and metabolic syndrome (11, 12). Recent studies used computed tomography (CT) imaging to identify and quantify visceral and subcutaneous fat, which is more accurate than BMI and waist circumference (WC) (13–15). Some studies showed that visceral fat area (VFA) $\geq 100\,cm^2$ is associated with metabolic syndrome and is a risk factor for poor prognosis and prolonged hospital stay after colorectal surgery (16).

Another concept that needs attention is sarcobesity (SO). Sarcopenia, which can occur independently of adiposity, is associated with physical disability, injuries, and mortality in individuals with non-malignant disease (17, 18). SO, the simultaneous occurrence of visceral obesity and low muscle mass, represents a worst-case scenario because it combines the health risks of visceral obesity and depleted lean mass (19). Pedrazzani et al. (20) reported that SO is a risk factor for developing cardiac complications and prolonged postoperative ileus (PPOI) after laparoscopic resection for colorectal cancer (CRC). The aim of this study was to evaluate the effects of VFA and SO on the incidence of total and surgical complications after radical resection of CRC.

Materials and methods

In this retrospective cohort study, we collected a total of 426 patients who underwent elective radical resection of CRC at Beijing Friendship Hospital, Capital Medical University from January 2017 to May 2018. All procedures were performed by experienced surgeons and their teams. The study protocol was approved by the Ethics committee of Beijing Friendship Hospital, Capital Medical University, with the number "2022-P2-104-01."

Inclusion criteria included: (1) age > 18 years; (2) an abdominal CT scan within 30 days before surgery; (3) only elective radical resection of colorectal cancer; (4) complete clinical data; and (5) complete postoperative pathological data of colorectal cancer. Exclusion criteria included: (1) absence or inability to obtain an abdominal CT scan within 30 days before surgery; (2) combined with other organ resection; (3) incomplete clinical data; (4) palliative surgery or emergency surgery; and (5) previous history of other malignancies. Patients without available or detailed CT images ($n = 32$) were excluded. Patients who underwent combined evisceration ($n = 4$) were excluded. One patient had a history of breast cancer, one had a history of thyroid cancer, and one had a history of ovarian cancer. Finally, 387 patients were included in our retrospective study.

Data collection

The following data were collected and analyzed retrospectively: (1) clinical data, including age, sex, smoking history, diabetes mellitus (DM), Charlson comorbidity index (CCI) (21), American Society of Anesthesiology (ASA) score, history of abdominal operation, neoadjuvant therapy, cancer (colon cancer or rectal cancer), operation (open, laparoscopic or convert to open), stoma, hemoglobin (HGB), albumin (ALB), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), fasting glucose, cholesterol, carcinoembryonic antigen (CEA), carbohydrate antigen199 (CA199), operative time, vascular invasion, and TNM classification; (2) postoperative complications: postoperative complications were graded according to the Clavien-Dindo Classification (CDC) (22) and divided into surgical complications (SC) and medical complications (MC). Surgical complications included anastomotic leakage (AL), wound infection, bleeding, abdominal infection, bladder dysfunction, intestinal obstruction, and rectovaginal fistula. Medical complications included cardiologic complications, respiratory complications, sepsis, and urinary tract infection (UTI). CDC $\geq$ III was defined as major complications (23).

CT-based quantification of body composition

In this study, all patients underwent abdominal CT scans within 30 days before surgery at Beijing Friendship Hospital, Capital Medical University. A scan at the level of the L3-L4 intervertebral disk was selected to measure the values of visceral fat area (VFA) and subcutaneous fat area (SFA) (14). VFA and SFA were identified and quantified using Hounsfield units (HU) thresholds of −150 to −50 HU and −190 to −30 HU, respectively (24). We defined VFA as the intra-abdominal adipose tissue area within the parietal peritoneum, excluding the paraspinal muscle, intervertebral bodies, and intramuscular fat. SFA was defined as the adipose tissue external to the peritoneum and back muscle (25). Total fat area (TFA) was defined as the sum of VFA and SFA. Visceral fat area to subcutaneous fat area ratio (V/S) was also calculated to assess the degree of visceral obesity.

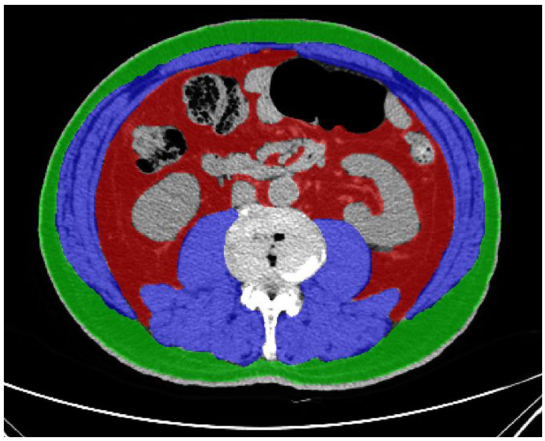

FIGURE 1
Cross sectional CT imaging at L3-L4 intervertebral disk. Muscle tissue is shown in blue color, subcutaneous fat tissue in green color, and visceral fat tissue in red color. Visceral fat tissue and subcutaneous fat tissue were identified and quantified using Hounsfield units (HU) thresholds of −150 to −50 HU and−190 to −30 HU. Skeletal muscle was identified and quantified by use of HU thresholds (−29 to +150).

Skeletal muscle was identified and quantified by use of HU thresholds (−29 to +150) (19). Skeletal muscle area (SMA) contains psoas, paraspinal muscles (erector spinae, quadratus lumborum), and abdominal wall muscles (transversus abdominus, external and internal obliques, and rectus abdominus). SO was defined using the VFA/SMA ratio (Sarcobesity Index). Because the standard cutoff values for VFA and SO were not uniform, we analyzed them as continuous variables.

Fat and skeletal muscle areas were labeled and measured semiautomatically by two experienced radiologists using ITK-SNAP version 3.8.0 (Figure 1).

Statistical analysis

We describe categorical variables as numbers with percentages. Continuous variables were described as means with SDs or medians with interquartile ranges, depending on the data distribution. VFA, SFA, TFA, V/S, and sarcobesity index were treated as continuous variables. Clinical variables were compared using independent samples *t* test, Pearson's chi-square test or Mann–Whitney U test as appropriate. Two continuous variables were evaluated using Spearman's rank correlation coefficient. LASSO regression was used to select the variables affecting the occurrence of postoperative complications. Multivariate analysis was performed for the selected variables with multiple logistic regression analysis. To increase the interpretability of the results, subgroup analysis was performed according to sex. In the subgroup analysis, LASSO regression and multiple logistic regression analysis were performed for male/female individually for variable selection and multivariate analysis. A receiver operating characteristic (ROC) curve analysis was used to develop a cut-off for sarcobesity index associated with postoperative outcome in males/females. And the cut-off values for sarcobesity index that maximized the Youden index (sensitivity + specificity −1) were defined as optimal. The patients were divided into control group and SO group according to the cut-off value of sarcobesity index. All of the tests were two-sided and considered statistically significant at $p < 0.05$. SPSS version 26.0 and R version 4.1.0 were used for statistical analysis. "glmnet" package was used to perform the LASSO regression. SPSS was used to perform multiple logistic regression analysis and ROC curves.

Results

Patient characteristics

387 adult patients underwent elective radical resection of CRC and met inclusion and exclusion criteria. Demographics and operative characteristics were shown in Table 1. The median age was 64 years, median BMI was 23.18 kg/m^2, 246 (63.6%) patients were female, and 141 (36.4%) patients were male. There were 247 (63.8%) patients with colon cancer and 140 (36.2%) patients with rectal cancer. 104 (26.9%) patients underwent open surgery, 255 (65.9%) patients underwent laparoscopic surgery, and 28 (7.2%) patients underwent laparoscopic conversion to open surgery.

Association between body composition and postoperative complications

128 (33.1%) patients developed complications, and 44 (11.4%) patients developed major complications (Table 2). Among them, 111 patients developed SC and 21 developed medical complications. The incidence of postoperative total complications was significantly associated with VFA, TFA, V/S, and sarcobesity index. Among them, surgical complications was significantly associated with VFA and V/S, but anastomotic leakage was only significantly associated with V/S. For medical complications and major complications, the differences in VFA, SFA, TFA, V/S, and SO were statistically significant.

Variables selection and multivariate analysis

To identify independent risk factors for total complications and surgical complications among the different variables measured, all available clinical indicators, including clinicopathological features, were subjected to LASSO regression (Figure 2). Further disciplinary regression was performed to take sarcobesity index, smoking, stoma, blood loss, operative time, and ASA as factors for postoperative total complications (Figure 3). Multiple Logistic regression analysis was then performed and showed that smoking, ASA ≥ III, increased sarcobesity index, and operative time were independent risk factors for postoperative total complications (Table 3). Same procedure was used to screen for independent risk factors for surgical complications (Table 4). The results showed that sarcobesity index, smoking, operative time were independent risk factors, and cholesterol was an independent protective factor for surgical complications. Smoking, increased sarcobesity index and operative time, and decreased cholesterol levels were associated with increased risk of surgical complications.

TABLE 1 Patients' characteristics.

Characteristics	Number of cases (*n*=387)
Age (years)	64 (58,72)
Sex	
Female	246 (63.6)
Male	141 (36.4)
BMI (kg/m^2)	23.18 (21.3,25.69)
Smoking	142 (36.7)
Diabetes mellitus	81 (20.9)
CCI	
≤2	356 (92.0)
>2	31 (8.0)
Abdominal surgery	98 (25.3)
Neoadjuvant	40 (10.3)
ASA score	
<III	208 (53.7)
≥III	179 (46.3)
Cancer	
Colon cancer	247 (63.8)
Rectal cancer	140 (36.2)
TNM stage	
<III	229 (59.2)
≥III	158 (40.8)
Surgery	
Open	104 (26.9)
Laparoscope	255 (65.9)
Convert to open	28 (7.2)
Stoma	111 (28.7)
Blood loss (mL)	50 (50,100)
Operative time (min)	230 (170,290)
Lymph node	15 (11,20)
Vascular invasion	157 (40.6)
TG (mmol/L)	1.19 (0.89,1.52)
HDL-C (mmol/L)	1.01 (0.84,1.21)
Glucose (mmol/L)	5.15 (4.74,5.74)
Cholesterol (mmol/L)	4.29 (3.73,4.94)
CEA (ng/mL)	3.14 (1.72,8.91)
CA199 (kU/L)	10.85 (5.40,22.50)
HGB (g/L)	122 (103,135)
Albumin (g/L)	36.9 (33.9,39.3)

The measurement data were expressed by median (interquartile ranges), and the enumeration data were expressed by the number of cases (percentage). CCI, Charlson comorbidity index; ASA score, American Society of Anesthesiology classification system score; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; CEA, carcinoembryonic antigen; CA199, carbohydrate antigen199; and HGB, hemoglobin.

Nomogram was constructed to predict total complications based on factors determined by multiple logistic regression analysis (Figure 4). ROC analysis was performed to determine the accuracy of logistic regression model. Area under the ROC curve (AUC) was 0.702 (Figure 5).

Variables selection and multivariate analysis of male patients for subgroup analysis

Because body composition was clearly different between males and females, subgroup analyses were performed according to sex. A total of 141 male patients were included in this study. All available clinical measures, including clinicopathological features and all obesity-related measures, were subjected to LASSO regression (Supplementary Figure 1; Figure 2). Finally, sarcobesity index, albumin, ASA score, stoma, and blood loss were selected as the factors of postoperative total complications, and then these factors were analyzed by multiple Logistic regression analysis. Multivariate analysis showed that albumin level was an independent protective factor for postoperative total complications in male patients (Table 5). The decrease of albumin increased the risk of postoperative total complications in male patients. ROC analysis was used to determine the accuracy of the logistic regression model. The AUC was 0.779 (Figure 6). A cutoff value of sarcobesity index≥1.395 cm^2, selected for maximizing Youden's J statistics, was associated with 46.2% sensitivity and 75.5% specificity.

Variables selection and multivariate analysis of female patients for subgroup analysis

A total of 246 female patients were included in this study. Smoking, operative time, cholesterol, and sarcobesity index were selected as the factors of postoperative total complications in female patients after LASSO regression (Supplementary Figure 3; Figure 4). Multiple Logistic regression analysis showed that smoking, operative time, and sarcobesity index were independent risk factors, and cholesterol was an independent protective factor for total postoperative complications in female patients (Table 6). Smoking, increased operative time and muscle mass, and decreased cholesterol levels increased the risk of overall postoperative complications in female patients. ROC analysis was used to determine the accuracy of the logistic regression model. The AUC was 0.707 (Figure 7). In the case of maximum Youden's J statistic, the cut-off value of muscle adiposity index was ≥0.845 cm^2, with a sensitivity of 71.9% and a specificity of 42%.

Comparison of clinical characteristics between control group and SO group

Sarcobesity was defined as sarcobesity index ≥1.395 cm^2 in male patients and ≥0.845 cm^2 in female patients. The patients were divided into SO group and control group. The differences in clinical characteristics between the control and SO groups were shown in Table 7. Patients had a higher age [66 (59,74) vs. 62 (55,70) years, $Z = -3.642$, $p < 0.001$], more diabetes mellitus (30.3 vs. 11.1%, $\chi^2 = 21.521$, $p < 0.001$), more stoma (33.3 vs. 23.8%, $\chi^2 = 4.288$,

TABLE 2 The correlation of sarcobesity index, visceral fat area (VFA), subcutaneous fat area (SFA), total fat area (TFA), ratio of visceral fat area to subcutaneous fat area (V/S), and postoperative complications.

		VFA		SFA		TFA		V/S		Sarcobesity index	
	n	t/Z	*p*	t/Z	*p*	t/Z	*p*	t/Z	*p*	t/Z	*p*
Total complication	128	-3.271	0.001	−0.757	0.449	−2.613	0.009	−2.633	0.008	−2.282	0.023
Surgical complications	111	−2.119	0.034	−0.136	0.892	−1.538	0.124	−2.01	0.044	−0.969	0.332
Anastomotic leakage	26	−1.307	0.191	−0.983	0.326	−0.673	0.501	−2.21	0.027	−0.804	0.421
Bleeding	20	−0.256	0.798	−1.123	0.262	−0.857	0.391	−0.139	0.89	−0.049	0.961
Abdominal infection	35	−1.994	0.046	−1.299	0.194	−2.084	0.037	−1.687	0.092	−1.587	0.113
Wound infection	15	−0.364	0.716	−0.82	0.412	−0.73	0.465	−0.234	0.815	−0.364	0.716
Intestinal obstruction	24	−1.777	0.076	−0.688	0.492	−0.91	0.363	−2.708	0.007	−1.513	0.13
Bladder dysfunction	7	−0.474	0.636	−0.91	0.363	−0.743	0.457	−0.218	0.827	−0.658	0.51
Rectovaginal fistula	1	−1.41	0.159	−0.103	0.918	−1.025	0.305	−1.589	0.112	−1.705	0.088
Medical complications	21	−3.734	<0.001	−1.397	0.162	−3.246	0.001	−2.738	0.006	−4.068	<0.001
Cardiologic complications	9	−2.406	0.016	−1.595	0.111	−2.408	0.016	−1.119	0.263	−2.457	0.014
Respiratory complications	7	−1.91	0.056	−0.984	0.325	−1.742	0.081	−1.156	0.248	−2.576	0.01
UTI	1	−1.41	0.159	−0.103	0.918	−1.025	0.305	−1.589	0.112	−1.705	0.088
Acute kidney injury	1	−1.41	0.159	−0.103	0.918	−1.025	0.305	−1.589	0.112	−1.705	0.088
Sepsis	3	−2.453	0.014	−1.062	0.288	−2.161	0.031	−2.166	0.03	−1.407	0.159
Major complication	44	−3.406	0.001	−0.276	0.783	−2.235	0.025	−3.473	0.001	−2.951	0.003

UTI, urinary tract infection; VFA, visceral fat area; SFA, subcutaneous fat area; TFA, total fat area; and V/S, visceral fat area to subcutaneous fat area ratio.

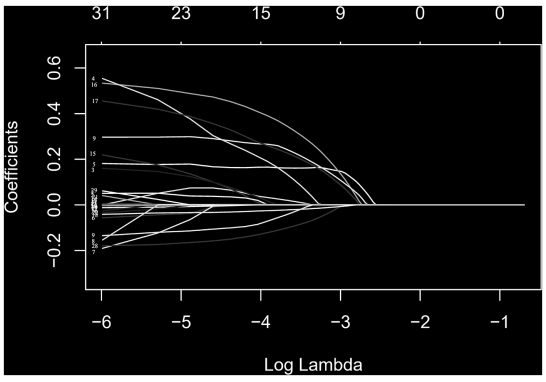

FIGURE 2
LASSO coefficient profiles of the 31 risk factors affecting the occurrence of postoperative total complications in patients with colorectal cancer. The risk factors corresponding to the number of each curve are shown in Supplementary Table 2.

$p = 0.038$), less harvested lymph nodes [14 (10, 19) vs. 16 (12, 22), $Z = -3.548$, $p < 0.001$], more blood loss [50 (50,100) vs. 50(50,150), $Z = -2.428$, $p = 0.015$] and more vascular invasion (46.4 vs. 34.9%, $\chi^2 = 5.276$, $p = 0.022$) in the SO group. However, there was no significant difference in smoking, CCI, cancer, neoadjuvant, TNM stage, operative time, and surgical methods between the two groups ($p > 0.05$). Multiple tests were used to correct for statistical differences among the three surgical procedures, and the results suggested that there was no statistically significant difference in surgical approach between the control and SO groups (Supplementary Table 1).

Discussion

This retrospective cohort study revealed that increased sarcobesity index is an independent risk factor for postoperative total complications in female patients, while visceral fat was not an independent risk factor for postoperative total complications in either male or female patients. Our findings suggest that sarcobesity, the

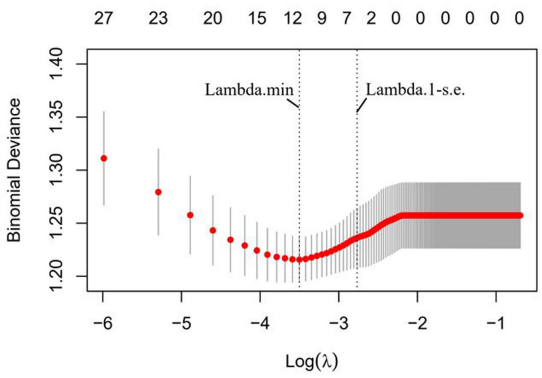

FIGURE 3
Six risk factors selected using LASSO Cox regression analysis. The two dotted vertical lines were drawn at the optimal scores by minimum criteria and 1-SE criteria (at 1-SE criteria including sarcobesity index, smoking, stoma, blood loss, surgery time, and ASA≥III).

TABLE 3 Multivariate analysis between total complications and risk/protective factors.

Parameters	B	SE	Wald	OR (95%CI)	*p*
Sarcobesity index	0.512	0.209	5.986	1.669 (1.107,2.515)	0.014
Smoking	0.589	0.237	6.165	1.803 (1.132,2.871)	0.013
Stoma	0.469	0.272	2.971	1.599 (0.938,2.726)	0.085
Blood loss	0.002	0.001	3.794	1.002 (1.000,1.004)	0.051
Operative time	0.003	0.002	4.222	1.003 (1.000,1.006)	0.040
ASA ≥ III	0.539	0.235	5.243	1.715 (1.081,2.720)	0.022

The parameters involved in the multivariate analysis were screened by LASSO regression. ASA, American Society of Anesthesiology classification system.

TABLE 4 Multivariate analysis between surgical complications and risk/protective factors.

Parameters	B	SE	Wald	OR (95%CI)	*p*
Sarcobesity index	0.436	0.209	4.372	1.547 (1.028,2.328)	0.037
Smoking	0.754	0.245	9.482	2.125 (1.315,3.432)	0.002
Blood loss	0.001	0.001	1.204	1.001 (0.999,1.002)	0.273
Operative time	0.004	0.001	9.265	1.004 (1.002,1.007)	0.002
Cholesterol	−0.381	0.14	7.438	0.683 (0.519,0.898)	0.006

The parameters involved in the multivariate analysis were screened by LASSO regression.

simultaneous occurrence of visceral obesity and low muscle mass, could be a better predictor of postoperative complications in CRC than visceral obesity at least in females. Several studies have compared the associated of visceral obesity and sarcobesity with the outcome of CRC surgery (20, 26). However, all of these were studies of European patients, who have different prevalence of obesity, obesity criteria, and skeletal muscle content than Asian populations (27, 28). In our study, only 10 patients (2.6%) met the criteria for obesity (BMI ≥ 30 kg/m^2), compared with 20–40% of the European population (29). In addition, these studies have a drawback of assessing sarcobesity index as a dichotomous variable, which assumes a predetermined cutoff value of sarcobesity index to define sarcobesity, when in fact, no consensus cutoff value exists. It is worth mentioning that many studies have used different criteria to define visceral obesity for males and females, while few studies have developed separate criteria to define SO according to sex. This may be the first study to investigate the association of VFA and sarcobesity index with postoperative complications of CRC in an Asian population.

Our study found that VFA was significantly associated with total complications, surgical complications, medical complications, and major complications ($p < 0.05$), but was not an independent risk factor for total complications and SC. Other studies have reached different conclusions (5, 9, 15). Watanabe et al. (5) reported that visceral obesity (cutoff value of 100 cm^2) independently predicted the incidence of overall postoperative complications of laparoscopic surgery for colon cancer ($p = 0.007$). This difference in results may be due to our inclusion of open surgery. Surgeons tend to prefer open surgery in patients with high visceral fat. Different cutoff values of VFA may be another reason for the difference (30, 31). Frostberg et al. (32) reported that VFA (cutoff value of 130 cm^2) were unable to predict complications after CRC surgery, contrary to the results of Watanabe et al. (5). Furthermore, we abandoned multivariate analysis of major complications and anastomotic leakage, because the number of these complications was too small and could easily lead to bias. By ROC analysis, we obtained the best cutoff value of sarcobesity index. We found SO patients had a lower number of lymph node dissection and a higher rate of conversion to open surgery. The College of American Pathologists has established guidelines for the pathologic evaluation of colorectal cancer resection specimens and recommended that a minimum of 12 lymph nodes should be removed (33). In three studies, improved survival was observed with more than 12 lymph nodes evaluated (34–36). The first quartile of the number of dissected lymph nodes [14 (10,19)] in the SO group exceeded the cutoff value. This means that more than a quarter of the patients in the SO group were unable to meet the requirements. The difference of dissected lymph nodes can have two explanations: the lymph nodes are located deep in the perivascular fat, and the large amount of visceral fat may affect the adequate resection of the lymph nodes (37); adipose tissue adheres to the mesentery, making it difficult for pathologists to identify lymph nodes (38). In our study, the SO group had more stomas and more intraoperative blood loss, but the operative time was not statistically different from the control group. We venture to hypothesize that advances in surgical techniques and devices may have markedly reduced the effect of large amounts of visceral fat on procedural time. However, the large amount of visceral adipose tissue still significantly increases the amount of intraoperative blood loss and the rate of stoma. This may be because separating large amounts of adipose tissue and exposing vital structures inevitably causes more

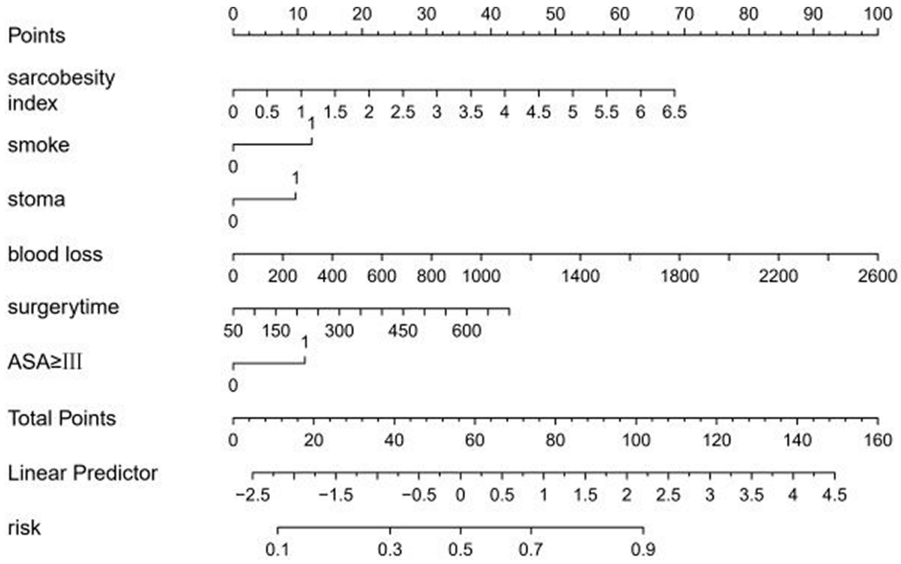

FIGURE 4
Nomogram including sarcobesity index (SO), smoking, stoma, blood loss, surgery time, and ASA≥III for total complications after radical resection of colorectal cancer.

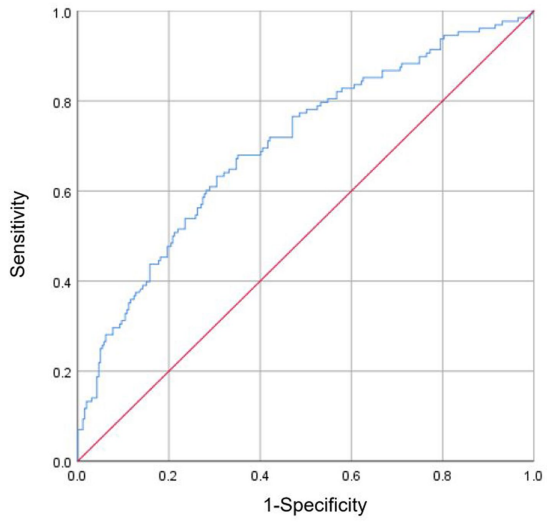

FIGURE 5
Receiver operating characteristic (ROC) analysis was performed to determine the reliability of the nomogram model for predicting the occurrence of total complications after radical resection of colorectal cancer. Area under the ROC curve (AUC) was 0.702.

bleeding and makes the surgeon more inclined to choose a prophylactic stoma.

In our study, we perceived an interesting finding that the proportion of females was significantly higher than that of males in the SO group (78.3 vs. 21.7%, $\chi^2 = 37.916$, $p < 0.001$). However, in several studies based on European populations, sarcobesity group had a high proportion of males (19, 20, 26). The fact that these studies did not define SO separately for males and females may be one reason for this discrepancy. Besides, VFA and SMA, two parameters that make up the formula for sarcobesity index, have sex differences. In our study, Asian females seem to have relatively more skeletal muscle loss or visceral fat accumulation. This may be due to the fact that Asian populations have more visceral fat compared to European populations (39). On the other hand, relatively low intake of red meat and impaired skeletal muscle mass in postmenopausal females may also contribute to sarcobesity in elder females (40, 41). Sarcobesity is an independent risk factor for postoperative complications of colorectal cancer in female patients. Therefore, it is of great significance to pay attention to this group of elderly females and improve their skeletal muscle loss and visceral fat accumulation before surgery.

Multivariate analysis showed that serum albumin and cholesterol were independent protective factors for postoperative complications in male and female patients, respectively. Preoperative serum albumin is well-known as an effective predictor of the outcome of colorectal cancer surgery and a component of nutritional screenings, such as the Prognostic Nutritional Index and Nutritional Risk Index (42–44). Hypoalbuminemic patients (serum albumin <35 g/L) are reported to have significantly higher rates of postoperative morbidity and mortality, as well as complications related to wounds and anastomosis compared with patients with normal serum albumin levels (45). For serum cholesterol, Lee et al. (46) found that the increase of serum cholesterol was related to a better outcome in patients undergoing gastrointestinal surgery. Low serum cholesterol could cause reduced lipopolysaccharide binding and neutralization, a reduced number of circulating lymphocytes, limited tissue repair and regeneration, and dysfunction of the hypothalamic–pituitary–adrenal axis (47–49). To a certain extent, serum albumin and cholesterol can reflect the nutritional status of patients (45, 47). It is suggested that strengthening perioperative nutritional status may improve the short-term outcome of patients with colorectal cancer.

The present study has the following limitations: because this study was a retrospective cohort study, the authors had to rely on accurate records from the treating physicians. The incidence of rare postoperative complications is low and the confidence interval is large.

TABLE 5 Multivariate analysis between total complications and risk/protective factors of male patients.

Parameters	B	SE	Wald	OR (95%CI)	*p*
Sarcobesity index	0.430	0.39	1.215	1.54 (0.72,3.30)	0.27
Albumin	−0.148	0.054	7.426	0.86 (0.78,0.96)	0.006
ASA ≥ III	0.798	0.477	2.791	2.22 (0.87,5.66)	0.095
Stoma	0.540	0.484	1.247	1.72 (0.67,4.43)	0.264
Blood loss (mL)	0.003	0.002	1.073	1.00 (1.00,1.01)	0.300

The parameters involved in the multivariate analysis were screened by LASSO regression. ASA, American Society of Anesthesiology classification system.

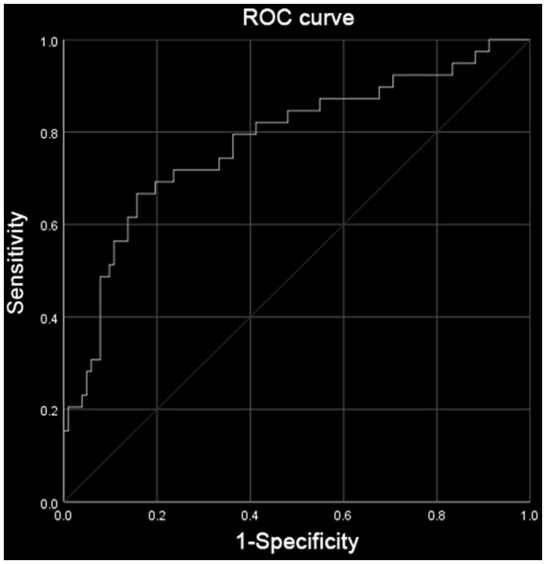

FIGURE 6
Receiver operating characteristic (ROC) analysis was performed to determine the reliability of the nomogram model for predicting the occurrence of total complications after radical resection of colorectal cancer in male patients. The area under the ROC curve (AUC) was 0.779.

TABLE 6 Multivariate analysis between total complications and risk/protective factors of female patients.

Parameters	B	SE	Wald	OR (95%CI)	*p*
Smoking	0.703	0.293	5.745	2.02(1.14,3.59)	0.017
Operative time	0.006	0.002	13.973	1.01(1.00,1.01)	<0.001
Cholesterol	−0.361	0.170	4.520	0.70(0.50,0.97)	0.033
Sarcobesity index	0.607	0.273	4.953	1.84(1.08,3.13)	0.026

The parameters involved in the multivariate analysis were screened by LASSO regression.

As noted above, *p* values represent descriptive, exploratory summary measures of comparison only and do not represent confirmatory test results. Besides, this study measured visceral fat, subcutaneous fat, and skeletal muscle tissue area but not volume. This could lead to inaccurate estimates.

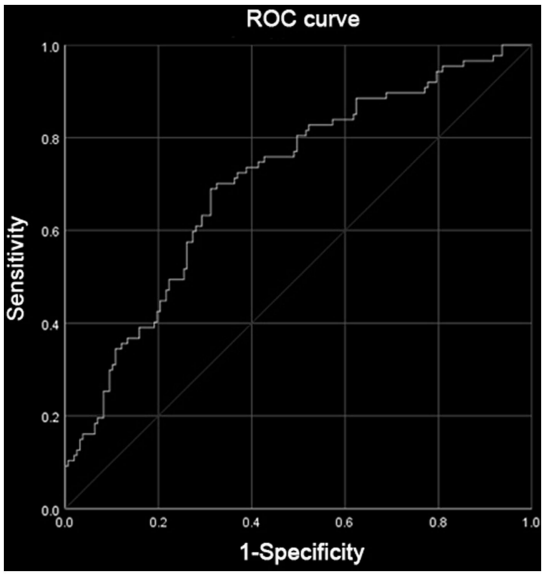

FIGURE 7
Receiver operating characteristic (ROC) analysis was performed to determine the reliability of the nomogram model for predicting the occurrence of total complications after radical resection of colorectal cancer in female patients. The area under the ROC curve (AUC) was 0.707.

Conclusion

Increased sarcobesity index is an independent risk factor for postoperative complications in patients with colorectal cancer, while visceral fat area is not. For female patients, smoking, operation time, and obesity index are independent risk factors for postoperative complications, while cholesterol is an independent protective factor. For male patients, serum albumin is an independent protective factor for postoperative complications. The number of dissected lymph nodes in sarcobesity patients was lower than that in general patients. Surgeons should therefore dissect lymph nodes more carefully in patients with sacrobesity, and be alert to the occurrence of postoperative complications in female patients with sacrobesity.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding authors.

Ethics statement

The studies involving human participants were reviewed and approved by Bioethics Committee of Beijing Friendship Hospital, Capital Medical University. The patients/participants provided their written informed consent to participate in this study.

TABLE 7 Differences in patient characteristics and postoperative pathological outcomes between sarcobesity group (SO) and control group.

	Control group (n=189)	SO group (n=198)	Z/χ^2	p
Age	62(55,70)	66(59,74)	−3.642	<0.001
Sex			37.916	<0.001
Female	91(48.1%)	155(78.3%)		
Male	98(51.9%)	43(21.7%)		
Smoking	62(32.8%)	80(40.4%)	2.404	0.121
DM	21(11.1%)	60(30.3%)	21.521	<0.001
CCI			2.405	0.121
≤2	178(94.2%)	178(89.9%)		
>2	11(5.8%)	20(10.1%)		
Cancer			0.119	0.730
Colon cancer	119(63.0%)	128(64.6%)		
Rectal cancer	70(37.0%)	70(35.4%)		
Neoadjuvant	17(9.0%)	23(11.6%)	0.717	0.397
TNM stage			3.594	0.058
<III	121(64.0%)	108(54.5%)		
≥III	68(36.0%)	90(45.5%)		
Stoma	45(23.8%)	66(33.3%)	4.288	0.038
Lymph nodes	16(12,22)	14(10,19)	−3.548	<0.001
Blood loss (mL)	50(50,100)	50(50,150)	−2.428	0.015
Operative time (min)	220(160,290)	240(180,290)	−1.605	0.108
Vascular invasion	66(34.9%)	91(46.4%)	5.276	0.022
Surgery[a]			5.282	0.071
Open	50(26.5%)	54(27.3%)		
Laparoscope	131(69.3%)	124(62.6%)		
Convert to open	8(4.2%)	20(10.1%)		

The measurement data were expressed by median (interquartile ranges), and the enumeration data were expressed by the number of cases (percentage). SO was defined as sarcobesity index ≥ 1.395 cm^2 in male patients and ≥ 0.845 cm^2 in female patients. DM, diabetes mellitus; CCI, Charlson comorbidity index.

[a]Multiple testing correction was used, as detailed in Supplementary Table 1.

Author contributions

ZF contributed to conception and design of the study and performed the statistical analysis. KP and MT wrote the sections of the manuscript. XG, HL, and XY organized the database. YY and ZZ contributed to conception and design of the study. All authors contributed to the article and approved the submitted version.

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Conflict of interest

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.

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2023.1126127/full#supplementary-material

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Association of longitudinal changes in skeletal muscle mass with prognosis and nutritional intake in acutely hospitalized patients with abdominal trauma: a retrospective observational study

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Background: The objective of this study was to explore whether longitudinal changes in skeletal muscle mass, from hospital admission to 3 weeks post-trauma, are associated with poor prognosis and nutritional intake in acutely hospitalized patients with abdominal trauma.

Methods: A single-center retrospective observational review was conducted on 103 patients with abdominal trauma admitted to the Affiliated Jinling Hospital, Medical School of Nanjing University between January 2010 and April 2020. Skeletal muscle mass was assessed by abdominal computed tomography (CT) performed within 14days before surgery and on post-trauma days 1–3 (week 0), 7–10 (week 1), 14–17 (week 2), and 21–24 (week 3). The skeletal muscle index (SMI) at L3, change in SMI per day (ΔSMI/day), and percent change in SMI per day (ΔSMI/day [%]) were calculated. The receiver-operating characteristic (ROC) curve was used to evaluate the discriminatory performance of ΔSMI/day (%) for mortality. Linear correlation analysis was used to evaluate the associations between ΔSMI/day (%) and daily caloric or protein intake.

Results: Among the included patients, there were 91 males and 12 females (mean age±standard deviation 43.74±15.53years). $\Delta SMI_{4\text{-}1}$/d (%) had a ROC-area under the curve of 0.747 (p =0.048) and a cut-off value of −0.032 for overall mortality. There were significant positive correlations between $\Delta SMI_{4\text{-}1}$/d (%) and daily caloric intake and protein intake (Y=0.0007501*X − 1.397, R^2 =0.282, R =0.531, p <0.001; Y=0.008183*X - 0.9228, R^2 =0.194, R =0.440, p <0.001). Δ SMI/day (%) was positively correlated with daily caloric intake ≥80% of resting energy expenditure in weeks 2, 3, and 1–3 post-trauma and with protein intake >1.2g/kg/d in weeks 3 and 1–3 post-trauma.

Conclusion: Loss of skeletal muscle mass is associated with poor prognosis and nutritional intake in patients admitted to hospital with abdominal trauma.

KEYWORDS

skeletal muscle index, nutrition, prognosis, muscle, trauma

1. Introduction

The prevalence of malnutrition ranges from 7 to 76% in severely injured patients (1). Malnutrition in hospitalized patients has been associated with adverse outcomes, including increased length of hospital stay, morbidity, in-hospital mortality, and cost (1, 2). The Global Leadership Initiative on Malnutrition criteria were established to identify malnutrition in the clinical setting, but there is no gold standard index for diagnosing malnutrition in clinical practice (3). Malnutrition is assessed based on parameters such as body weight, body mass index (BMI), skinfold thickness, and body composition analysis. Accurate diagnosis of malnutrition is affected by post-traumatic edema, serosal effusion, becoming bedridden, and clinician awareness.

Critically ill patients must be provided adequate nutritional support to prevent metabolic deterioration and loss of skeletal muscle mass, which negatively impacts clinical outcomes. Low skeletal muscle mass is an independent predictor of poor clinical prognosis in patients with abdominal trauma (4), and evaluation of the cross-sectional area of the psoas major muscle at the third lumbar vertebral level (L3) has been used as a surrogate measure of decreased strength and functional capacity in older adults (2, 3, 5, 6). Computed tomography (CT) assessment of skeletal muscle mass can be used to quantitatively monitor muscle loss. Coupling monitoring of longitudinal changes in skeletal muscle mass with actively supervised nutritional support programs may be beneficial to the prognosis of critically ill or trauma patients.

The objective of the present study was to explore whether longitudinal changes in skeletal muscle mass, from hospital admission to 4 weeks post-trauma, are associated with poor prognosis and nutritional intake in acutely hospitalized patients with abdominal trauma. Our hypothesis is that longitudinal changes in skeletal muscle are associated with prognosis in trauma patients, and we then test this hypothesis using the receiver-operating characteristic (ROC) curves. Furthermore, we confirm the correlation between nutrition intake and longitudinal changes in skeletal muscle.

2. Methods

This study was performed at the Research Institute of General Surgery, Affiliated Jinling Hospital, Medical School of Nanjing University. The protocol was approved by the Hospital Ethics Review Board on November 2, 2021 (Approval #: 2021NZKY-045-01). Our unit is a national trauma center, it is also an abdominal trauma center, with 46 ICU beds, and about 200 trauma patients are admitted every year, and about 150 trauma patients require surgery.

2.1. Study design and population

A single-center retrospective observational study included patients with abdominal trauma who admitted to the Affiliated Jinling Hospital, Medical School of Nanjing University between January 2010 and April 2020. Abdominal trauma is defined as blunt or penetrating injury to the abdominal cavity. The abdominal cavity upper limit extends from the horizontal plane passing through the base of the xiphoid process and the spinous process of the 12th dorsal vertebra. It is situated between the cephalad side of the thoracic cavity and the caudal side of the pelvis. The pubic symphysis marks the beginning of the lower boundary of the abdominal cavity, which continues along the entire inguinal arc and iliac crest, and terminates at the spinous process of the 5th lumbar vertebra (7). Inclusion criteria were: (1) age 18–80 years; (2) length of hospital stay >30 days; (3) abdominal CT performed within 14 days before surgery and on post-trauma days 1–3 (week 0), 7–10 (week 1), 14–17 (week 2), and 21–24 (week 3). Exclusion criteria were: (1) missing data; (2) discharge within 72 h of hospitalization; (3) pregnancy; (4) history of mental illness.

2.2. Data collection

The medical records of included patients were retrospectively reviewed. Baseline demographic and clinical characteristics were recorded at hospital admission, including sex, age, body weight, BMI (ratio of body weight [kg] to height [m^2]), trauma type, vital signs at arrival, injured organ, readmission, abbreviated injury scale (AIS), injury severity score (ISS), route of nutrition, nutritional risk screening (NRS), serum levels of albumin, and mechanical ventilation, vasopressor support and transfusion. Clinical chemistry included leukocyte count, C-reactive protein (CRP), procalcitonin (PCT), albumin, and transferrin. The age-adjusted Charlson comorbidity index (ACCI) was used to evaluate comorbidity.

Skeletal muscle mass was assessed by abdominal CT. The skeletal muscle index (SMI) at L3 was calculated as skeletal muscle area (SMA) (cm^2)/height (m^2) at hospital admission and post-trauma week 0, week 1, week 2, and week 3. Skeletal muscle was demarcated using predetermined thresholds: −29 to +150 Hounsfield units (HU) for muscle tissue, −150 to −50 HU for visceral adipose tissue, and − 190 to −30 HU for subcutaneous and intramuscular adipose tissue (Neusoft, China). Cut-off values of the SMI for low skeletal muscle mass were 42.08 cm^2/m^2 for men and 37.35 cm^2/m^2 for women, according to our previously published study (4).

Clinical outcomes included 30-day mortality, 60-day mortality, 90-day mortality, length of hospital stay, hospital costs, and use of mechanical ventilation, continuous renal replacement therapy (CRRT), vasopressors, transfusion and/or laparotomy.

2.3. Definitions

Abdominal CT performed during post-trauma weeks 0–3 was denoted as CT1, CT2, CT3, and CT4, respectively.

Skeletal muscle area and SMI at L3 measured on abdominal CT performed during post-trauma weeks 0–3 were denoted as SMA_1, SMA_2, SMA_3 and SMA_4 and SMI_1, SMI_2, SMI_3 and SMI_4, respectively (Figure 1).

Changes in SMA or SMI were calculated as $\Delta SMA_{2-1} = SMA_2 - SMA_1$, $\Delta SMA_{3-2} = SMA_3 - SMA_2$, $\Delta SMA_{4-3} = SMA_4 - SMA_3$ and $\Delta SMA_{4-1} = SMA_4 - SMA_1$, or $\Delta SMI_{2-1} = SMI_2 - SMI_1$, $\Delta SMI_{3-2} = SMI_3 - SMI_2$, $\Delta SMI_{4-3} = SMI_4 - SMA_3$ and $\Delta SMI_{4-1} = SMI_4 - SMI_1$.

Change in SMA or SMI per day was calculated as ΔSMA = (post SMA – pre SMA)/ days between initial abdominal CT scan and final abdominal CT scan, or ΔSMI = (post SMI – pre SMI)/ days between initial abdominal CT scan and final abdominal CT scan.

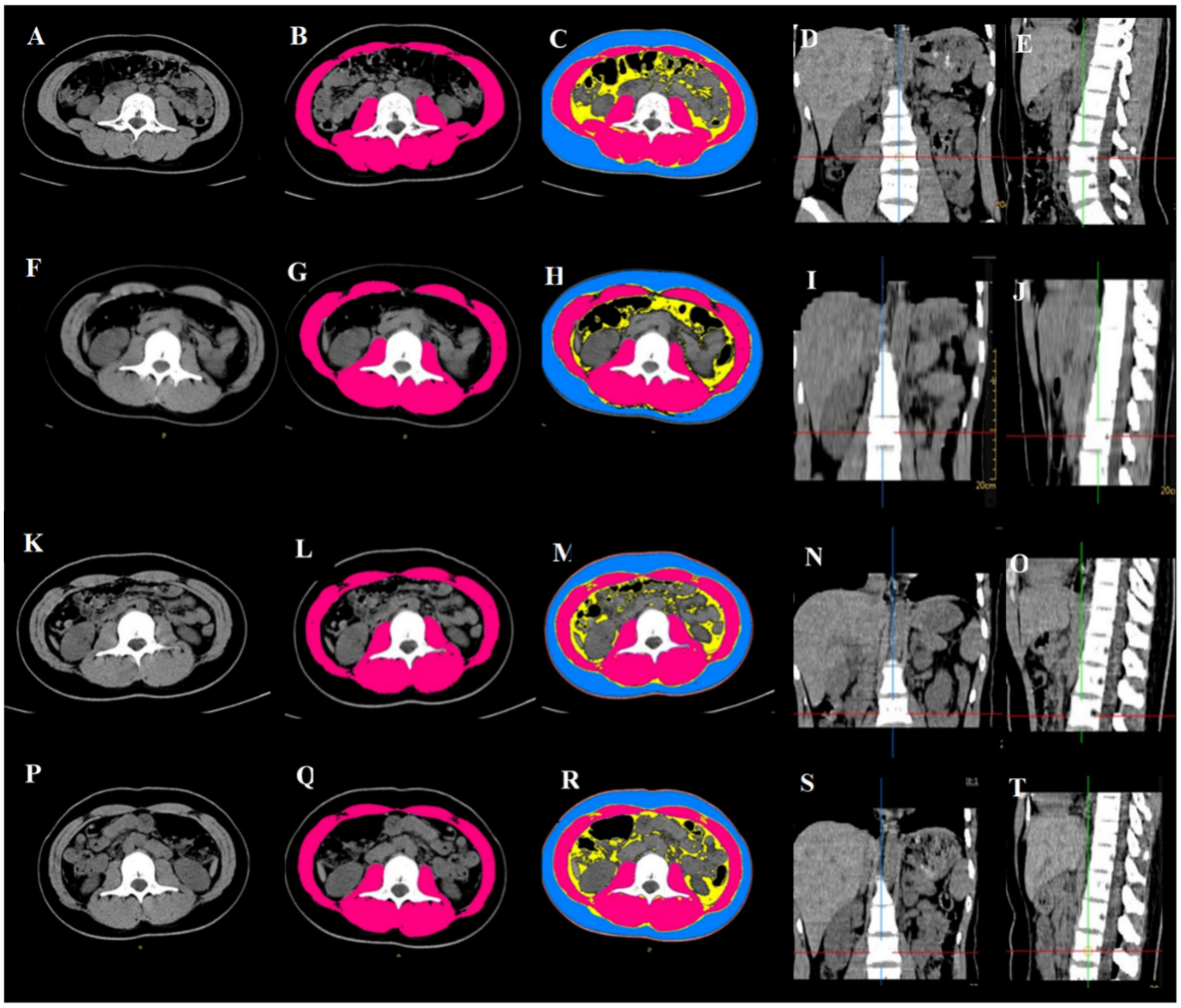

FIGURE 1
Abdominal CT scan showing changes in skeletal muscle mass at L3 after abdominal trauma in an 18-year female. **(A–E)** Within 14days before surgery: SMA=104cm^2, SMI=38.81cm^2/m^2; **(F–J)** post-trauma week 1: SMA=83.5cm^2, SMI=31.42cm^2/m^2; **(K–O)** post-trauma week 2: SMA=81.22cm^2, SMI=31.42cm^2/m^2; **(P–T)** post-trauma week 3: SMA=78.03cm^2, SMI=29.36cm^2/m^2. **(A,F,K,P)** horizontal cross section at L3; **(B,G,L,Q)**: skeletal muscle mass at L3 (red); **(C,H,M,R)** subcutaneous fat (blue), abdominal fat (yellow), skeletal muscle (red); **(D,I,N,S)** coronal plane, L3 is at the intersection of the red line and blue line; **(E,J,O,T)** saggital plane, L3 is at the intersection of the red line and green line.

Percent change in SMA (ΔSMA/day [%]) or SMI (ΔSMI/day [%]) per day was calculated as ΔSMA/day (%) = (SMA at final abdominal CT scan – SMA at initial abdominal CT scan)/SMA at initial abdominal CT scan × 100/days between initial abdominal CT scan and final abdominal CT scan or ΔSMI/day (%) = (SMI at final abdominal CT scan – SMI at initial abdominal CT scan)/SMI at initial abdominal CT scan × 100/days between initial abdominal CT scan and final abdominal CT scan. This approach was adapted from previous studies that have calculated Δ SMI/year (%) (8, 9) and Δ SMI/ month (%) (10).

2.4. Nutritional therapy and measurement

Patients with abdominal trauma were provided clinical nutrition according to ESPEN guidelines. Low-calorie nutrition (not exceeding 70% of resting energy expenditure) was provided in the acute phase. The caloric intake was increased to 80–100% of resting energy expenditure and protein intake was 1.2–2.0 g /kg/d after 72 h.

2.5. Statistics

Statistical analyses were conducted using SPSS version 22 software (IBM, Inc., Armonk, NY, United States). Data are presented as mean ± SD or median and interquartile ranges for continuous variables and were compared using the *t*-test or Mann–Whitney *U* test, as appropriate. Categorical data are expressed as numbers and proportions and were compared using the χ^2 test or Fisher's exact test, as appropriate. Nonparametric tests, such as the Mann–Whitney *U* test or Wilcoxon test, were used for non-normally distributed data. Images were evaluated by three radiologists. We used Kendall's W consistency coefficient and intraclass correlation coefficients (ICC) to test the consistency of CT images and assess their quality. Kendall's *W*

was 0.834 ($p < 0.001$) and the ICC was 0.801 (95% confidence interval [CI], 0.758–0.829) ($p < 0.001$) (zero indicates no agreement between raters; 1 indicates perfect agreement), indicating a strong consistency between the three clinicians. The ROC curve was used to evaluate the discriminatory performance of ΔSMI/day (%) for mortality (area under the curve [AUC] > 0.9 high accuracy; 0.7–0.9 moderate accuracy; 0.5–0.7 low accuracy). Youden's index was used to determine the optimal cut-off value. Linear correlation analysis was used to evaluate the associations between ΔSMI/day (%) and daily caloric intake (caloric intake ≥80% of resting energy expenditure, 50–80% of resting energy expenditure, < 50% of resting energy expenditure) (11), and between ΔSMI/day (%) and daily protein intake (protein intake ≥2.0 g/kg/d, 1.2–2.0 g/kg/d, < 1.2 g/kg/d). $p < 0.05$ was considered statistically significant.

3. Results

3.1. Study population

A flow chart of the study participants is shown in Figure 2. A total of 1,112 patients with abdominal trauma were admitted to the Affiliated Jinling Hospital, Medical School of Nanjing University between January 2010 and April 2020. Of these, 990 patients had missing data, 15 patients were discharged within 3 days of admission, and 4 patients died within 1 day of admission. Finally, 103 patients were included in the analysis. The demographic and clinical characteristics of the included patients were summarized in Table 1. 88.3% ($n = 91$) of patients were males. 99 (96%) patients suffered blunt abdominal trauma, 4 (4%) patients suffered penetrating trauma, and 18 (17.5%) patients were readmissions.

3.2. Clinical outcomes

Clinical outcomes of the included patients are summarized in Table 2. Thirty-day, 60-day, and 90-day mortalities were 5.8, 6.8, and 7.8%, respectively. Thirty-nine (37.8%) patients required mechanical ventilation, and the median duration of mechanical ventilation was 18 days. Twelve (11.6%) patients required CRRT, and the median duration of CRRT was 7 days. Twenty-one (20.2%) patients required norepinephrine. Forty-one (39.8%) patients received blood transfusion, and 53 (51.4%) patients underwent laparotomy.

3.3. Skeletal muscle area and skeletal muscle index at L3

3.3.1. Skeletal muscle area and skeletal muscle index

Skeletal muscle area at L3 during post-trauma weeks 0–3 is shown in Figure 3A. SMA_3 and SMA_4 were significantly lower than SMA_1 (135.78 ± 24.83 vs. 152.63 ± 32.72 cm^2, $p < 0.001$; 132.83 ± 28.42 vs. 152.63 ± 32.72 cm^2, $p < 0.001$). SMA_4 was significantly lower than SMA_2 (132.83 ± 28.42 vs. 143.73 ± 30.42 cm^2, $p = 0.038$). SMI at L3 during post-trauma weeks 0–3 is shown in Figure 3B. SMI_3 and SMI_4 were significantly lower than SMI_1 (46.47 ± 7.92 vs. 52.33 ± 10.28 cm^2/m^2, $p < 0.001$, 45.90 ± 9.25 vs. 52.33 ± 10.28 cm^2/m^2, $p < 0.001$).

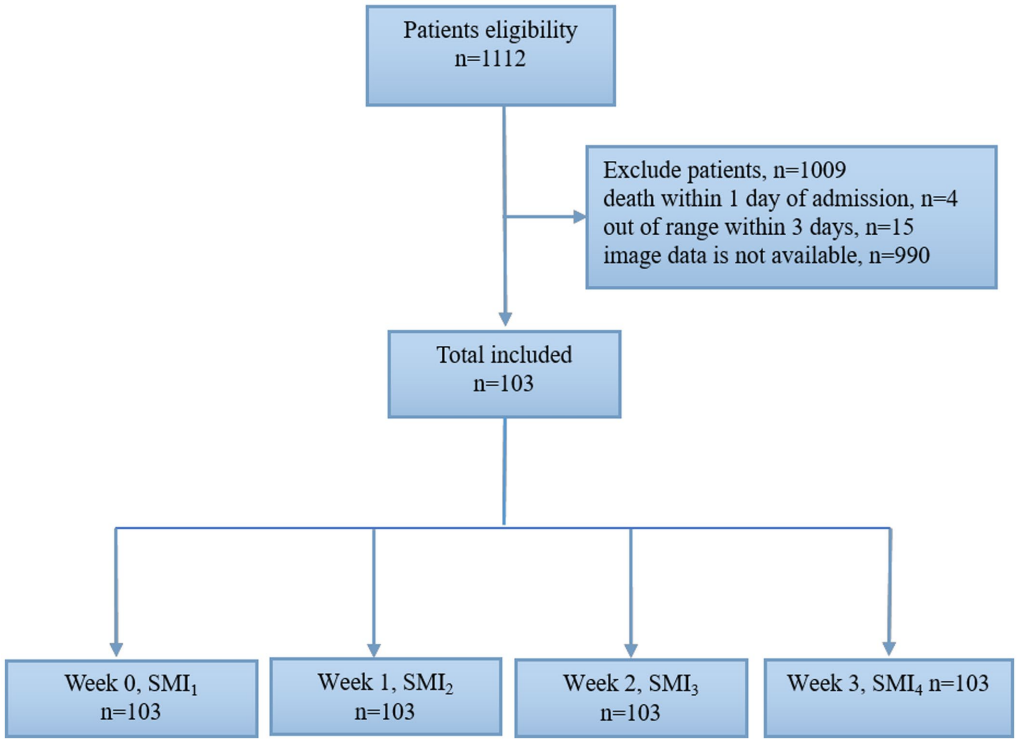

FIGURE 2
Study flow chart.

TABLE 1 Demographic and clinical characteristics of the included patients.

Characteristics	Value
Age, y, Mean ± SD	43.74 ± 15.53
Sex, n (%)	
Male	91 (88.3)
Female	12 (11.7)
Weight, kg, Mean ± SD	66.89 ± 11.58
BMI, kg/m^2, Mean ± SD	22.85 ± 3.36
ACCI score, Median (IQR)	1 (0–2)
Type of injury, n (%)	
Blunt	99 (0.96)
Penetrating	4 (0.04)
Vital signs at arrival, Median (IQR)	
GCS	14 (13–15)
HR	82 (72–98)
RR	17 (15–20)
SBP	123 (109–130)
Injured organs, n (%)	
Gastric	1 (0.9)
Duodenal	2 (1.8)
Small intestine	16 (15.5)
Colon	3(2.9)
Mesentery	4 (3.9)
Liver	15 (14.6)
Spleen	6 (5.8)
Pancreas	6 (5.8)
Multiple abdominal Injuries	50 (48.5)
Readmission, *n* (%)	18 (17.5)
ISS, n (%)	
15–24	27 (26.2)
25–34	33 (32.0)
35–44	6 (5.8)
≥45	5 (4.8)
ISS ≥ 16, *n* (%)	71 (68.9)
AIS HEAD score ≥ 3, *n* (%)	18 (17.5)
AIS THORAX score ≥ 3, *n* (%)	52 (50.5)
AIS ABDOMEN score ≥ 3, *n* (%)	77 (74.7)
AIS EXTREMITIES score ≥ 3, *n* (%)	16 (15.5)
Route of nutrition, n (%)	
Enteral nutrition	9 (0.0)
Parenteral nutrition	19 (24.0)
Combined enteral and parenteral nutrition	59 (60.0)
None	16 (16.0)
Leukocyte (◊10^9/L), Mean ± SD	14.02 ± 6.73
CRP (mg/L), Mean ± SD	27.76 ± 12.43
PCT (Mean ± SD)(ng/mL), Mean ± SD	3.53 ± 0.77
NSR 2002, Mean ± SD	3.49 ± 1.67
Albumin (g/L), Mean ± SD	31.3 ± 5.66
Transferrin (g/L), Mean ± SD	1.76 ± 0.54

SD, standard deviation; BMI, body mass index; ACCI, age-adjusted Charlson comorbidity index; GCS, Glasgow coma score; SBP, systolic blood pressure; RR, respiratory rate; ISS, injury severity score; AIS, abbreviated injury scale; APACHE-II, acute physiology and chronic health evaluation II; CRP, C-reactive protein; PCT, procalcitonin; NRS, nutritional risk screening.

3.3.2. ΔSMA/d (%) and ΔSMI/d (%)

ΔSMA/d (%) at L3 is shown in Figure 3C. $\Delta SMA_{2\text{-}1}$/d (%) showed a significantly greater decrease than $\Delta SMA_{4\text{-}3}$/d (%) (median [IQR] −0.99 [−2.05–0.11] vs. −0.28 [−1.00–0.18], $p < 0.01$). $\Delta SMA_{3\text{-}2}$/d (%) was significantly greater than $\Delta SMA_{4\text{-}3}$/d (%) (median [IQR] −0.65 [−1.57 to 0.00] vs. −0.28 [−1.00–0.18], $p < 0.01$). $\Delta SMA_{4\text{-}1}$/d (%) was significantly greater than $\Delta SMA_{3\text{-}2}$/d (%) (median [IQR] −0.87 [−1.84 to 0.26] vs. −0.65 [−1.57 to 0.00], $p < 0.01$) and $\Delta SMA_{4\text{-}3}$/d (%) (median [IQR] -0.87 [−1.84 to 0.26] vs. −0.28 [−1.00 to 0.18], $p < 0.01$).

ΔSMI/d (%) is shown in Figure 3D. $\Delta SMI_{2\text{-}1}$/ d (%) showed a significantly greater decrease than $\Delta SMI_{4\text{-}3}$/d (%) and $\Delta SMI_{4\text{-}1}$/d (%) (median [IQR] −0.91 [−1.94–0.07] vs. −0.33 [−1.09–0.03], $p < 0.01$; −0.91 [−1.94–0.07] vs. −0.47 [−0.89–0.00], $p < 0.01$).

3.4. ΔSMI/d (%) as a prognostic predictor

Receiver-operating characteristic curve analysis examining the predictive value of Δ $SMI_{4\text{-}1}$/d (%) for overall mortality is shown in Table 3 and Figure 4. Δ $SMI_{4\text{-}1}$/d (%) had a ROC-AUC of 0.747 ($p = 0.048$) and a cut-off value of −0.032 (Table 3).

3.5. Nutritional therapy

Daily caloric intake were 641.27 ± 335.02 kcal/d, 1274.76 ± 686.77 kcal/d, 1334.39 ± 815.83 kcal/d, 1212.02 ± 489.70 kcal/d, respectively in post-trauma week 1, week 2, week 3, week 1–3. Daily protein intake were 17.35 ± 8.97 g/d, 47.38 ± 24.83 g/d, 66.86 ± 28.34 g/d, 44.36 ± 29.63 g/d, respectively in post-trauma week 1, week 2, week 3, week 1–3.

3.6. ΔSMI/d (%) and daily caloric intake and protein intake

$\Delta SMI_{2\text{-}1}$/d (%), $\Delta SMI_{3\text{-}2}$/d (%), $\Delta SMI_{4\text{-}3}$/d (%) were not correlated with daily caloric intake, and also $\Delta SMI_{2\text{-}1}$/d (%), $\Delta SMI_{3\text{-}2}$/d (%) with protein intake ($p > 0.05$). But there were significant positive correlations between $\Delta SMI_{4\text{-}3}$/d (%) and daily protein intake ($Y = 0.004583*X - 0.864$, $R^2 = 0.042$, $R = 0.205$, $p = 0.041$), $\Delta SMI_{4\text{-}1}$/d (%) and daily caloric intake and protein intake ($Y = 0.0007501*X - 1.397$, $R^2 = 0.282$, $R = 0.531$, $p < 0.001$; $Y = 0.008183*X - 0.9228$, $R^2 = 0.194$, $R = 0.440$, $p < 0.001$; Figure 5).

$\Delta SMI_{2\text{-}1}$/d (%) were not correlated with daily caloric intake ≥80%, 50–80%, or <50% of resting energy expenditure and protein intake ($p > 0.05$) (Figures 6A,B).

ΔSMI_{3-2}/d (%) was positively correlated with daily caloric intake ≥80% of resting energy expenditure ($Y = 0.0008021*X - 1.801$, $R^2 = 0.064$, $R = 0.253$, $p = 0.036$). It was not correlated with daily protein intake ($p > 0.05$) (Figures 6C,D).

ΔSMI_{4-3}/d (%) were positively correlated with daily caloric intake ≥80% of resting energy expenditure ($Y = 0.0007551*X - 1.992$, $R^2 = 0.073$, $R = 0.270$, $p = 0.044$) and protein intake >1.2 g/kg/d ($Y = 0.0217*X - 2.951$, $R^2 = 0.472$, $R = 0.687$, $p < 0.001$; $Y = 0.0288*X - 4.271$, $R^2 = 0.421$, $R = 0.649$, $p = 0.031$), and negatively correlated with daily caloric intake <50% of resting energy expenditure ($Y = -0.00253*X + 0.6439$, $R^2 = 0.156$, $R = 0.395$, $p = 0.028$) (Figures 6E,F).

ΔSMI_{4-1}/d (%) were positively correlated with daily caloric intake ≥80% of resting energy expenditure ($Y = 0.001142*X - 2.081$, $R^2 = 0.282$, $R = 0.531$, $p < 0.001$) (Figure 6G), and protein intake <1.2 g/kg/d, 1.2–2.0 g/kg/d and ≥2.0 g/kg/d ($Y = 0.00915*X$-09248, $R^2 = 0.084$, $R = 0.289$, $p = 0.007$; $Y = 0.0286*X - 2.471$, $R^2 = 0.602$, $R = 0.775$, $p = 0.024$; $Y = 0.03174*X - 4.513$, $R^2 = 0.432$, $R = 0.657$, $p = 0.028$) (Figure 6H).

The inflammatory response was evaluated in patients with a daily caloric intake <50% ($n = 29$) or ≥50% ($n = 74$) of resting energy expenditure. Findings showed that CRP and PCT levels were significantly higher in patients with a daily caloric intake <50% compared to ≥50% of resting energy expenditure ($p < 0.01$) (Figure 7).

TABLE 2 Clinical outcomes of the included patients.

Clinical outcomes	Value
30-day mortality, *n* (%)	6 (5.8)
60-day mortality, *n* (%)	7 (6.8)
90-day mortality, *n* (%)	8 (7.8)
Hospital LOS, d, Mean ± SD	35.46 ± 7.42
Hospital cost, $\times 10^4$$, Mean ± SD	2.54 ± 1.42
Mechanical ventilation, *n* (%)	39 (37.8)
Mechanical ventilation, d, Median (IQR)	18 (6–38)
CRRT, *n* (%)	12 (11.6)
CRRT, d, Median (IQR)	7 (3–17)
Vasopressor support, *n* (%)	21 (20.3)
Transfusion, *n* (%)	41 (39.8)
Transfusion volume, mL, Median (IQR)	1,535 (875–3,800)
Massive transfusion, >10 RBC units, *n* (%)	15 (14.6)
Laparotomy, *n* (%)	53 (51.4)

LOS, length of stay; CRRT, continuous renal replacement therapy; IQR, interquartile range.

4. Discussion

This study explored whether longitudinal changes in skeletal muscle mass, from hospital admission to 3 weeks post-trauma, are associated with poor prognosis and nutritional intake in acutely hospitalized patients with abdominal trauma. Findings showed a rapid decrease in skeletal muscle mass at L3 in week 1 post-trauma, and ΔSMI_{4-1}/d (%) was capable of predicting poor prognosis. ΔSMI/day (%) was an effective indicator of nutritional status, and increasing daily caloric intake to ≥80% of resting energy expenditure at 2 weeks post-trauma and daily protein intake to ≥1.2 g/kg/d at 3 weeks

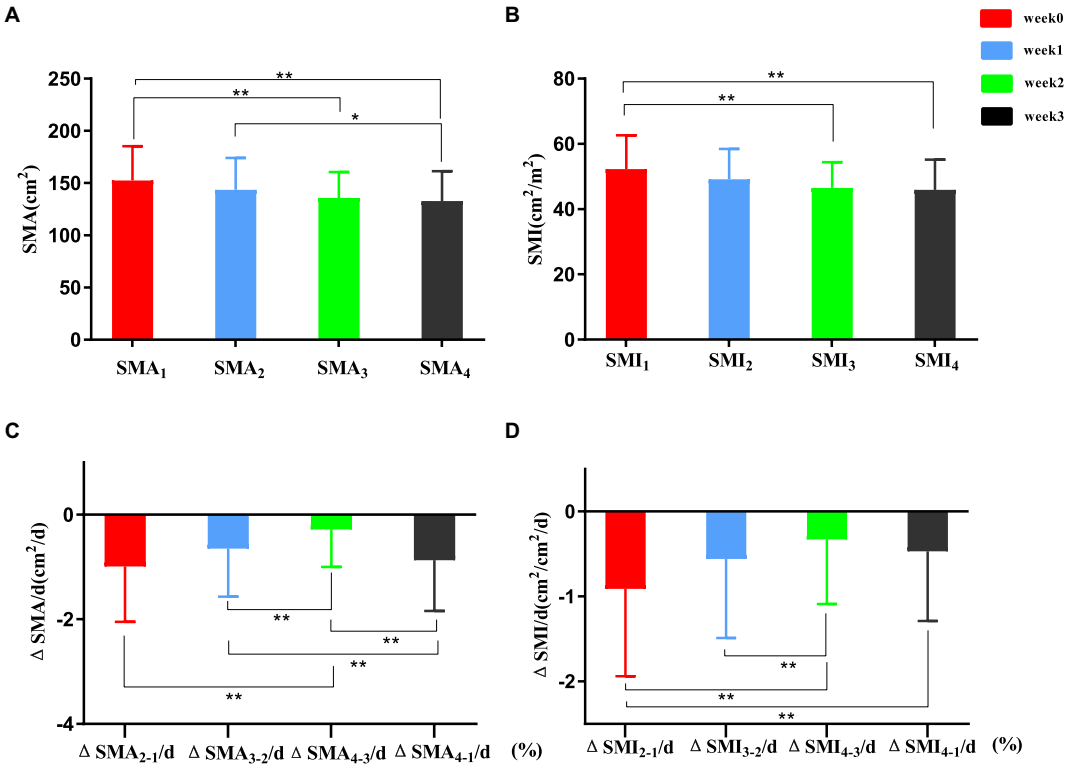

FIGURE 3
SMA **(A,C)** and SMI **(B,D)** at L3. Numbers are mean±SEM (n=103). *p<0.05, **p<0.01.

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TABLE 3 Receiver-operating characteristic (ROC) analysis examining the predictive value of ΔSMI/d (%) for mortality.

	AUC	95%CI value of *p*	Sensitivity		Specificity	Youden's Index	Cut-off
ΔSMI_{3-2}/d (%)	0.644	(0.459, 0.828)	0.249	100.0%	43.1%	0.431	−0.389
ΔSMI_{2-1}/d (%)	0.698	(0.467, 0.930)	0.112	5.0%	94.8%	0.448	0.469
ΔSMI_{4-3}/d (%)	0.454	(0.277, 0.631)	0.712	100.0%	24.1%	0.241	−0.599
ΔSMI_{4-1}/d (%)	0.747	(0.535, 0.959)	0.048*	50.0%	94.8%	0.448	−0.032

AUC, area under the curve. *$p < 0.05$.

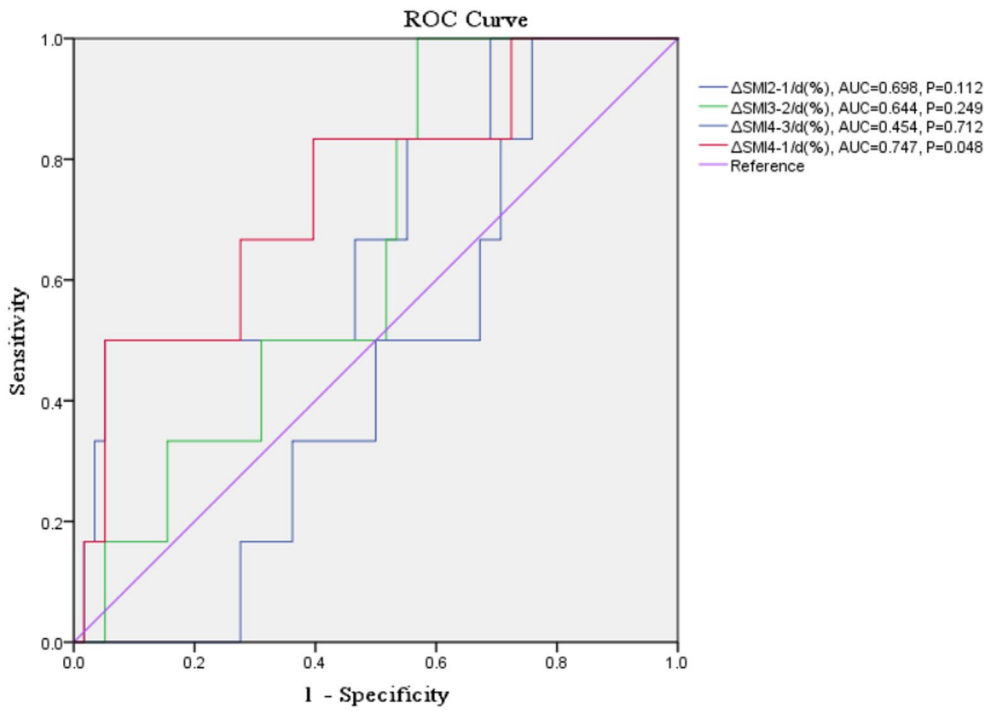

FIGURE 4
Receiver-operating characteristic (ROC) curve of ΔSMI/d (%) in predicting mortality.

post-trauma slowed depletion of skeletal muscle mass at L3. These results suggest skeletal muscle mass should be evaluated in the first week for abdominal trauma patients.

Malnutrition is an independent risk factor for complications, mortality, long hospital length of stay, and reduced quality of life in critically ill and trauma patients (1, 12, 13). Common indicators of malnutrition include weight, BMI, skin fold thickness, and body composition analysis; however, these indicators are highly variable and may not accurately represent nutritional status in all situations (14, 15). Weight and height are often determined from a subjective estimation in critically ill patients (16). Infection, bleeding, and edema affect body mass and body composition analysis. Severe stress and hypercatabolism during early trauma may affect resting energy consumption and a patient's metabolic rate. Currently, there are no standard methods for screening and diagnosing patients with malnutrition. CT evaluation of skeletal muscle is widely used in patients who are critically ill, have undergone surgery, or have cancer (17–19). Evaluation of progressive changes in skeletal muscle mass by CT may provide evidence of a patient's nutritional status.

In the present study, rates of change in SMA and SMI at L3 varied during the first 3 weeks post-trauma. SMA and SMI were most rapidly depleted in week 1 post-trauma. Accordingly, previous reports have shown that skeletal muscle mass decreases by 2–4% per day in patients in the ICU (20), patients with an ICU length of stay of 40 days can lose up to 40% of total body protein, mostly from skeletal muscle (21), and critically ill patients with intra-abdominal sepsis have an acute and persistent loss of muscle mass averaging an 8% decrease in SMI from baseline at 3 months (22). Skeletal muscle has been identified as a key metabolic and homeostatic organ (23). Muscle plays a central role in protein metabolism when dietary intake fails to meet the body's protein needs. Skeletal muscle is a reservoir of amino acids and a site where essential amino acids are synthesized. In trauma or other critical conditions, muscle breaks down to supply protein to vital tissues and organs (24). Short-term disuse atrophy (<10 days) is particularly relevant to the depletion of skeletal muscle mass (25). A retrospective study of mechanically ventilated critically ill patients who had an abdominal CT scan (including L3) between day 1 and day 4 after admission to the ICU showed that low SMA was a risk factor

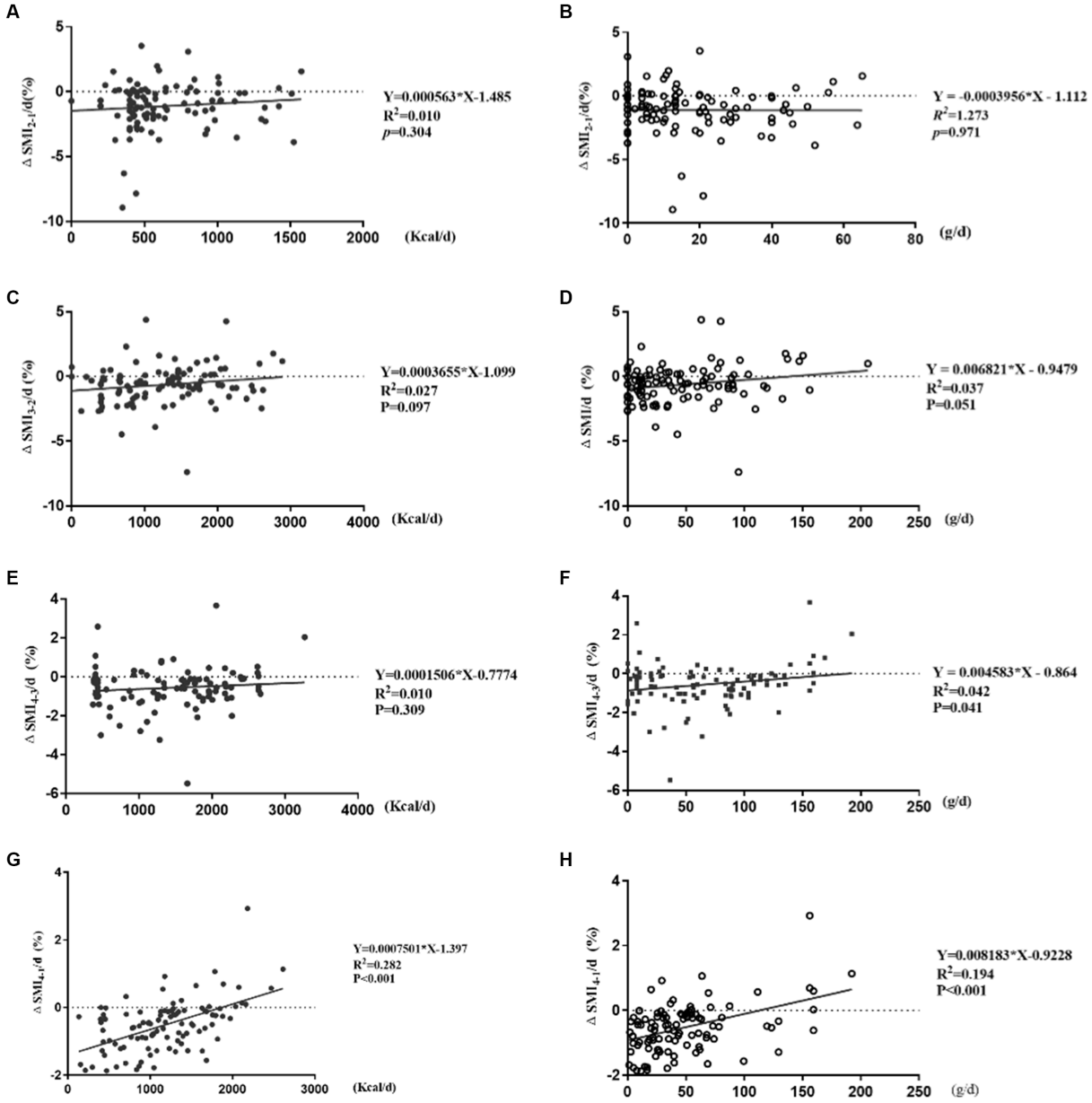

FIGURE 5
Correlation analysis of ΔSMI/d (%) and daily caloric and protein intake. **(A,B)** Correlation of $\Delta SMI_{2\text{-}1}$/d (%) and caloric and protein intake; **(C,D)** correlation of $\Delta SMI_{3\text{-}2}$/d (%) and caloric and protein intake; **(E,F)** correlation of $\Delta SMI_{4\text{-}3}$/d (%) and caloric and protein intake; **(G,H)** correlation of $\Delta SMI_{4\text{-}1}$/d (%) and caloric and protein intake.

for mortality (26). In another study of critically ill patients, muscle loss occurred early and rapidly during the first week of critical illness and was more severe among those with multi-organ failure compared with single organ failure (27). A prospective observational study of patients admitted to the medicine or cardiothoracic ICU with a diagnosis of sepsis or acute respiratory failure showed that skeletal muscle loss in the first 7 days of ICU admission can predict physical function at hospital discharge (28).

Preventative measures that enhance anabolism and reduce catabolism are required to reduce loss of skeletal muscle mass during the acute period of trauma. In the present study, Δ SMI/day (%) was used to investigate the time course of loss of skeletal muscle mass after abdominal trauma. Δ SMI/day (%) was the most severe in the first week (median Δ SMI $_{2\text{-}1}$/day (%) = − 0.91%), and Δ SMI/day (%) during 4 weeks post-trauma significantly predicted poor prognosis in our patient population. For comparison, the median Δ SMI/year (%) in cirrhotic patients was estimated as − 0.22% (8).

In the present study, approximately 50% of patients with a daily caloric intake of <50% of resting energy expenditure during week 1 post-trauma appear to consume more caloric and lose more skeletal muscle. These factors may be contributing to their condition, such as severe infection, unstable hemodynamics, serious complications, and co-morbidities. The robust inflammatory response in the acute stage of abdominal trauma in patients with a daily caloric intake of <50% of resting energy expenditure was reflected by higher CRP and PCT levels. Previously studies showed that reaching both protein and

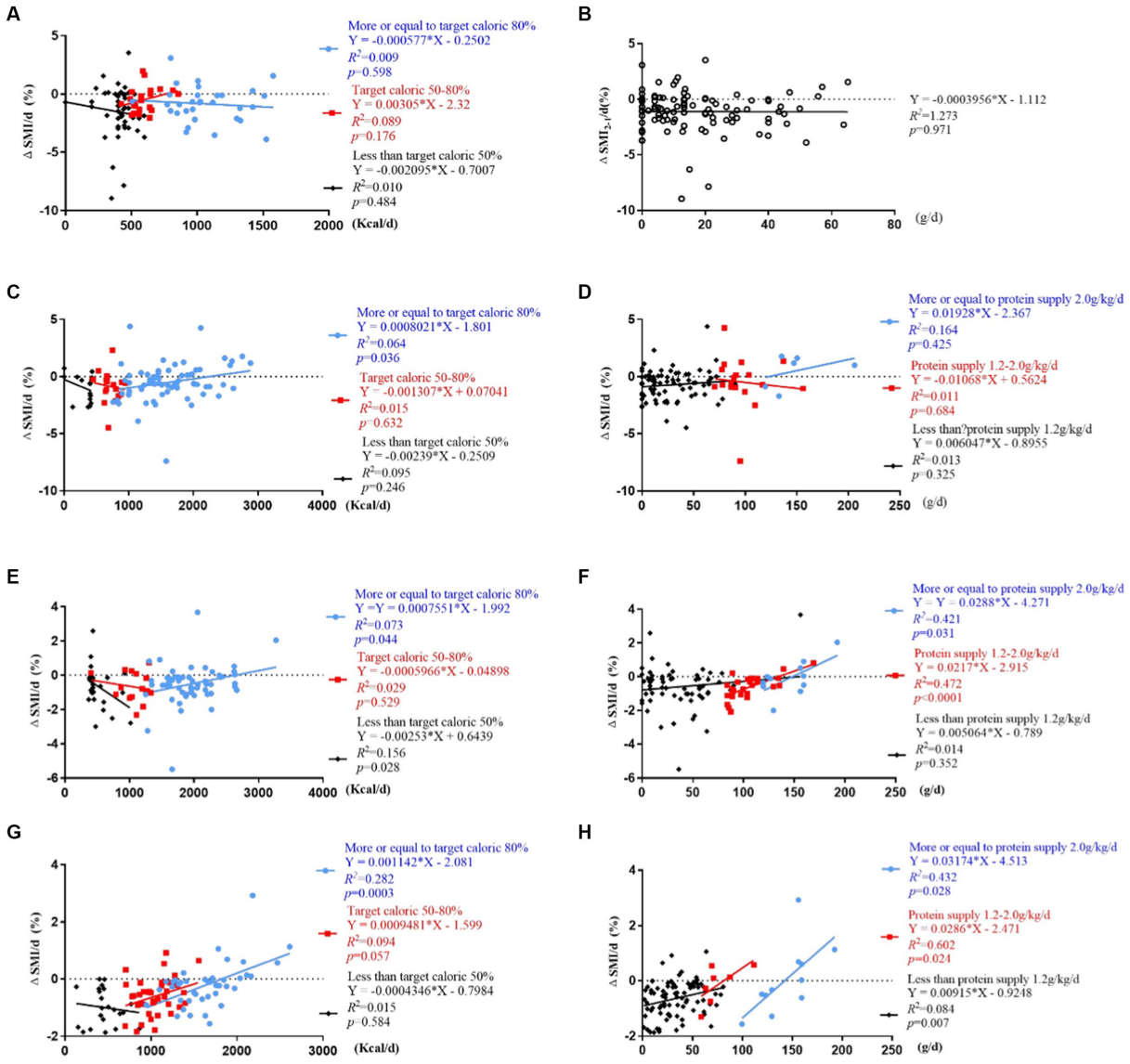

FIGURE 6
Correlation analysis of ΔSMI/d (%) and daily caloric and protein intake stratified by caloric (<50%, 50–80%≥80% of resting energy expenditure) and protein (<1.2g/kg/d, 1.2–2g/kg/d, ≥ 2g/kg/d) target levels. **(A,B)** Correlation of $\Delta SMI_{2\text{-}1}$/d (%) and caloric and protein intake; **(C,D)** correlation of $\Delta SMI_{3\text{-}2}$/d (%) and caloric and protein intake; **(E,F)** correlation of $\Delta SMI_{4\text{-}3}$/d (%) and caloric and protein intake; **(G,H)** correlation of $\Delta SMI_{4\text{-}1}$/d (%) and caloric and protein intake.

energy targets improves survival in critically ill patients (29, 30). A retrospective cohort study of patients hospitalized in an ICU showed underfeeding and overfeeding were harmful to critically ill patients, achieving a caloric target of 70% of resting energy expenditure had a survival advantage, and excessive caloric intake was associated with longer length of stay and length of ventilation (31). A multicenter cohort study of adult patients who were mechanically ventilated for more than 8 days in the ICU showed nutritional intake received during the first week in the ICU was associated with longer survival time and faster physical recovery to 3 months (11). Conversely, two large randomized controlled trials of critically ill patients reported no differences in clinical endpoints after low, normal, or high-calorie intake during early hospitalization in the ICU (32, 33).

Trauma induces complex metabolic changes (34) that involve a neuroendocrine component, release of gastrointestinal hormones, and an inflammatory/immune component. These changes have been associated with anorexia, sepsis, uncontrolled oxidative stress that can damage essential proteins, membrane lipids and DNA, and insulin resistance that can lead to uncontrolled catabolism of peripheral tissues such as fat and muscle (35). Trauma and its management often lead to loss of mobility, which accelerates decrease of skeletal muscle mass and strength.

In the present study, rate of loss of skeletal muscle mass decreased after the caloric target was increased to 80% of resting energy expenditure in post-trauma week 2. At this stage, muscle mass may be maintained by stable hemodynamics, progressive recovery of gastrointestinal function, improved organ function, reduced catabolism, and increased anabolism. Excessive calorie intake may not be associated with changes in skeletal muscle mass; rather, adequate provision of energy and protein is important in reducing muscle loss

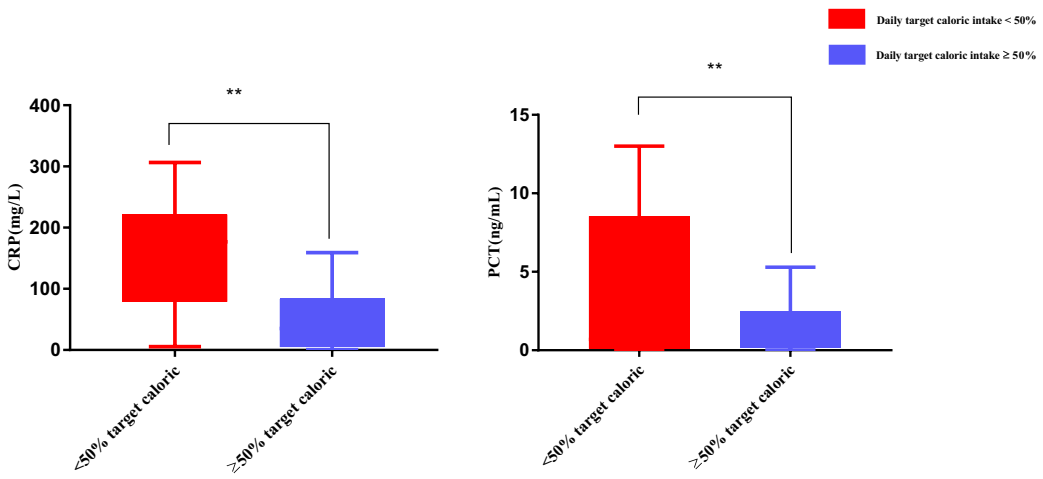

FIGURE 7
Inflammatory response in patients with a daily caloric intake <50% of resting energy expenditure. Numbers are mean±SEM (n=103). **p<0.01.

in critically ill patients (36). A prospective randomized study of patients staying in the ICU ≥ 5 days with outcomes recorded until day 90 showed daily caloric intake of 10–20 kcal/kg and protein intake of 0.8–1.2 g protein/kg, both approaching currently recommended targets, was associated with earlier weaning from invasive mechanical ventilation and longer survival compared to a daily intake above or below these values (37). A prospective, randomized, single-center, pilot clinical trial of mechanically ventilated patients in an adult ICU showed provision of an actively supervised nutritional intervention and providing near target energy requirements was associated with lower hospital mortality (38).

In the present study, ΔSMI/day (%) was positively correlated with daily protein intake during the 4 weeks post-trauma and increasing protein intake to >1.2 g/kg/d at 3 weeks post-trauma slowed depletion of skeletal muscle mass, suggesting that protein supplementation may reduce loss of skeletal muscle mass during the acute period of trauma. Protein is essential for the maintenance of muscle quality and function, and clinical guidelines indicate that critically ill patients may benefit from a protein intake of 1.2 g/kg/d (39). A high-protein intake (1.3 g/kg adjusted body weight/day) is recommended by the 2018 ESPEN guidelines for obese critically ill patients and 2021 ESPEN guidelines for patients with COVID19 (40, 41). In one study of critically ill patients, higher protein delivery in the first week was associated with greater loss of skeletal muscle mass (27). However, further research is needed to optimize recommendations of daily protein intake in acute trauma patients.

However, this study had several limitations. First, the single-center retrospective observational study design introduced the risk of bias and residual confounding. Skeletal muscle changes may be influenced by age, sex, ACCI, severity of trauma, and severity of infection, etc. Further multivariate regression analysis was necessary to eliminate bias due to a number of confounding factors. Second, because the research span is 10 years, the posttraumatic treatment concept may be updated, which may further bias the results. Third, the results were based on short-term follow-up in the hospital, and long-term outcomes were not assessed. Furthermore, the type of abdominal trauma may have influenced the results. Most importantly, nearly 90% of patients are excluded, which may cause bias. In future studies, we will rigorously calculate sample size estimates and develop more scientific inclusion and excluded criteria and follow-up systems. Finally, although this is a single-center, observational, retrospective study in Asia, the methodology can be applied to western populations as well. In the future, a multicenter prospective randomized controlled trial will be required to confirm the impact of nutritional support on abdominal trauma-related skeletal muscle quality. As a follow-up, we will conduct mathematical and computer modeling based on various factors (such as age, height, weight, and disease severity) to develop accurate dose formulas for nutritional supplements. This study will provide a new method for nutritional assessment and a new direction for nutritional treatment.

5. Conclusion

This study showed that skeletal muscle mass loss is associated with poor prognosis and nutritional intake in patients admitted to hospital with abdominal trauma. Skeletal muscle mass at L3 decreased rapidly in week 1 post-trauma. ROC curve analysis showed that Δ $SMI_{4\text{-}1}$/d (%) was predictive of clinical prognosis. Skeletal muscle changes were correlated with daily caloric and protein intake: there was a positive correlation between Δ SMI/day (%) and daily protein intake in week 3 post-trauma, and a positive correlation between Δ SMI/day (%) and daily caloric and protein intake in weeks 1–3 post-trauma. Δ SMI/day (%) was positively correlated with daily caloric intake ≥80% of resting energy expenditure in weeks 2, 3 and 1–3 post-trauma and with protein intake >1.2 g/kg/d in weeks 3 and 1–3 post-trauma. Evaluation of longitudinal changes in skeletal muscle mass may inform the development of nutritional support programs and improve prognosis in patients admitted to hospital with abdominal trauma.

Data availability statement

The raw data contributions presented in the study are included in the article/supplementary material, be directed to the corresponding authors.

Ethics statement

The protocol for this study was reviewed and approved by the Institutional Review Board of the Research Institute of General Surgery, Affiliated Jinling Hospital, Medical School of Nanjing University on November 2, 2021. Written informed consent for participation was not required for this study, according to national and institutional legislation, because it was a retrospective study based on the electronic medical record.

Author contributions

WY designed the study and performed the data analysis and interpretation. FX, YY, and WD collected the data and wrote the manuscript. ST carried out the study and data analysis. TG and YC helped to draft the tables and figures. WY and ST conceived the study, participated in its design and coordination, and helped draft the manuscript. All the authors critically reviewed the manuscript and approved the final version.

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Conflict of interest

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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Serum creatinine/cystatin C ratio as a prognostic indicator for patients with colorectal cancer

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Background: This study aimed to explore the relationship between creatinine/cystatin C ratio and progression-free survival (PFS) and overall survival (OS) in colorectal cancer (CRC) patients undergoing surgical treatment.

Methods: A retrospective analysis was conducted on 975 CRC patients who underwent surgical resection from January 2012 to 2015. Restricted three-sample curve to display the non-linear relationship between PFS/OS and creatinine-cystatin C ratio. Cox regression model and Kaplan-Meier method were used to evaluate the effect of the creatinine-cystatin C ratio on the survival of CRC patients. Prognostic variables with p-value ≤0.05 in multivariate analysis were used to construct prognostic nomograms. The receiver operator characteristic curve was used to compare the efficacy of prognostic nomograms and the traditional pathological stage.

Results: There was a negative linear relationship between creatinine/cystatin C ratio and adverse PFS in CRC patients. Patients with low creatinine/cystatin C ratio had significantly lower PFS/OS than those with high creatinine/cystatin C ratio (PFS, 50.8% vs. 63.9%, p = 0.002; OS, 52.5% vs. 68.9%, p < 0.001). Multivariate analysis showed that low creatinine/cystatin C ratio was an independent risk factor for PFS (HR=1.286, 95%CI = 1.007–1.642, p=0.044) and OS (HR=1.410, 95%CI=1.087–1.829, p=0.010) of CRC patients. The creatinine/cystatin C ratio-based prognostic nomograms have good predictive performance, with a concordance index above 0.7, which can predict the 1–5-year prognosis.

Conclusion: Creatinine/cystatin C ratio may be an effective prognostic marker for predicting PFS and OS in CRC patients, aid in pathological staging, and along with tumour markers help in-depth prognostic stratification in CRC patients.

KEYWORDS

creatinine/cystatin C ratio, nutrition, colorectal cancer, progression-free survival, overall survival

Introduction

Colorectal cancer (CRC) is the third and second most common cause of cancer morbidity and mortality, respectively, with nearly 2 million new cases and 1 million deaths reported worldwide in 2020 (1). CRC is the second most commonly diagnosed cancer and the fifth leading cause of cancer-related deaths in China (2). Surgical treatment remains the mainstay for CRC treatment. Social and economic development have led to increasingly improved methods for CRC treatment, including chemoradiotherapy, immunotherapy, traditional Chinese medicine treatment, and so on. Despite the progress in anti-tumour therapy for CRC, the long-term prognosis of CRC patients remains unsatisfactory, especially in patients with advanced CRC (3, 4). Therefore, it is necessary to find effective prognostic biomarkers to maximize the survival time of CRC patients.

At present, tumour-specific factors, such as pathological stage, perineural invasion, and vascular invasion, are the most commonly used tools for prognostic prediction, efficacy monitoring, and treatment formulation in CRC patients. However, due to their invasive nature, these tools have certain limitations. Additionally, owing to tumour heterogeneity, the prognosis of patients varies significantly within the same pathological stage (5, 6). Individual patient factors, including nutritional status, physical performance, and skeletal muscle mass, are also crucial to determine the prognosis of CRC patients. The detection and management of sarcopenia is a key aspect of the prognosis management in CRC patients. However, the diagnosis of sarcopenia requires device-dependent muscle mass measurements including dual-energy X-ray absorptiometry (DXA), computed tomography (CT), and bioelectrical impedance analysis (BIA), which are not widely used due to their high cost and radioactivity. Several studies have shown that straightforward, economical, and effective blood characteristics and their combinations can be used to accurately predict the clinical outcome of patients with cancer (7). Recent reports have suggested that the creatinine/cystatin C ratio in peripheral blood can be used to predict sarcopenia and prognosis in patients with cancer (8, 9). In 2017, Kashani et al. first developed the creatinine/cystatin C ratio and verified the correlation between creatinine/cystatin C ratio and sarcopenia (9). Since then, the creatinine/cystatin C ratio has been reported to be associated with sarcopenia and prognosis in various cancers (10–13). Serum creatinine and cystatin C are commonly used serum markers for evaluating glomerular filtration function in clinical practice (14). Serum creatinine is a derivative of the skeletal muscle protein creatine phosphate, which is mainly affected by the metabolism of muscle tissue *in vivo*; while, cystatin C, a small non-ionic protein derived exclusively from all nucleated cells and slightly metabolized by muscle tissue, may be used to estimate glomerular filtration function without concern for lean body mass and nutritional status (9, 15). Creatinine and cystatin C are derived from different cells. Creatinine reflects muscle metabolism, while cystatin C acts as a correction for renal function load. Therefore, the creatinine/cystatin C ratio may be a promising prognostic marker for CRC patients.

However, there is currently little research on the association between creatinine/cystatin C ratio and prognosis in CRC patients. Therefore, this study aimed to explore the relationship between creatinine/cystatin C ratio and progression-free survival (PFS) and overall survival (OS) in CRC patients undergoing surgical treatment, and develop a novel prognostic model based on creatinine/cystatin C ratio to accurately predict clinical outcomes.

Patients and methods

Study design

This study retrospectively included CRC patients who received surgical treatment in the Department of Colorectal and Anal Surgery, the First Affiliated Hospital, Guangxi Medical University from 2012 to 2015. The inclusion criteria were as follows: 1) patients diagnosed with CRC who received surgical treatment within a limited time; 2) serum creatinine, serum cystatin C, and other blood biochemical tests were performed within five days before surgery; 3) Patients with follow-up for at least 2 months; 4) Patients aged between 18 and 89 years old, with autonomy and no cognitive impairment. The exclusion criteria were as follows: 1) The primary site of the tumor is unclear or the tumor of multiple sources and multiple sites; 2) Patients with severe renal insufficiency or immune deficiency before surgery; 3) Patients who have received preoperative neoadjuvant radiotherapy or chemotherapy. This study strictly complied with the provisions of the Declaration of Helsinki and was approved by the Ethics Committee of the First Affiliated Hospital of Guangxi Medical University. Due to the retrospective study design, no informed consent was required.

Data collection

Demographic and laboratory data were retrieved from electronic databases and patient medical records. Baseline demographic data included age, sex, height, weight, body mass index (BMI), and comorbidity (hypertension and diabetes). BMI is defined as body weight (kg)/height squared (m^2). Pathological information included pathological stage, tumor stage (T stage), node stage (N stage), metastasis stage (M stage), perineural invasion, vascular invasion, pathological type, differentiation, tumor location, and maximum tumor size. Pathological staging adopted the TNM stage of the American Joint Committee on Cancer (AJCC) cancer staging 8th edition. Peripheral venous blood was collected from all patients after fasting for 8 hours in the week before surgery. Total blood count, serum creatinine concentration, serum cystatin C, and serum Carcinoembryonic antigen (CEA) were measured using an automated hematology analyzer (Beckman Coulter AU5800). The formula for calculating the serum creatinine/cystatin C ratio was as follows: (serum creatinine/serum cystatin C) × 100%.

Follow-up and outcome

The CRC patients who recovered well after surgery were followed up regularly after discharge. Follow-up included regular

visits to outpatient and inpatient clinics and telephone follow-up. The patients were followed up every 3 to 6 months in the first year and every 6 to 12 months starting in the second year until death. The main contents of the follow-up were inquiring about patients' basic living conditions, serum tumor marker examination, abdominal CT, and electronic fiber colonoscopy. PFS was defined as the time interval between the date of the patient's surgery and the patient's disease recurrence or death. OS was defined as the time interval between the date of the patient's surgery and death from any cause or the last follow-up. The last follow-up was on July 31, 2021.

Statistical analysis

R language version 4.0.2 (http://www.R-project.org) statistical software was used for statistical analysis. Measurement data are expressed as mean ± standard deviation and were compared by an independent sample t-test. Enumeration data are expressed as numbers (percentages) and compared using chi-square tests. The optimal layering method was used to determine the optimal cutoff value of the creatinine-cystatin C ratio. Restricted Cubic Splines (RCS) are used to explore the associations between the creatinine-cystatin C ratio and PFS/OS. Survival curves were estimated by the Kaplan-Meier method, and survival rates were compared by the Log-rank test. Univariate and multivariate COX regression models were used to evaluate the risk factors affecting prognosis in CRC patients. Prognostic variables with p-value ≤0.05 in multivariate analysis were included to construct prognostic nomograms, and their discriminant ability was evaluated by the Concordance index (C-index). In addition, the calibration curves were used to compare the predicted probabilities of these nomograms with the actual results through 1000 resampling. The receiver operator characteristic curve (ROC) was used to compare the efficacy of prognostic nomograms and traditional TNM staging in predicting the prognosis of CRC patients. Finally, the total population was randomly divided into the validation cohorts at a ratio of 7:3 for internal validation. In this study, a two-tailed p-value less than 0.05 was considered statistically significant.

Results

Clinicopathological characteristics of the study population

According to inclusion criteria and exclusion criteria, 975 patients were finally eligible to be included in this study. Baseline clinicopathologic characteristics were shown in Table 1. The mean age of CRC patients was 57.50 ± 13.14 years old. 821 (63.0%) patients were men. There were 496 (48.1%) patients in the I-II stage and 479 (49.1%) patients in the III-IV stage. There were 476 cases (48.8%) of rectal cancer and 499 cases (51.2%) of colon cancer. Serum CEA was elevated (≥5.0 U/mL) in 411(42.2%) patients. Perineural invasion occurred in 88 (9.0%) patients and vascular invasion occurred in 145 (14.9%) patients. The optimal critical value of the creatinine-cystatin C ratio for predicting the prognosis of CRC patients was 106.75

TABLE 1 The clinicopathological factors of CRC patients.

Clinicopathological characteristics	Overall (n = 975)	Surviving patients (n = 551)	Deceased patients (n = 424)	p value
Sex(Man)	607 (62.3)	347 (63.0)	260 (61.3)	0.644
Age (mean (SD))	57.50 (13.14)	56.55 (12.19)	58.73 (14.20)	0.01
BMI (median [IQR])	57.50 (13.14)	56.55 (12.19)	58.73 (14.20)	0.01
Hypertension (Yes)	147 (15.1)	77 (14.0)	70 (16.5)	0.314
Diabetes (Yes)	61 (6.3)	30 (5.4)	31 (7.3)	0.289
T stage (T3-4)	735 (75.4)	369 (67.0)	366 (86.3)	<0.001
N stage				<0.001
N0	529 (54.3)	358 (65.0)	171 (40.3)	
N1	288 (29.5)	152 (27.6)	136 (32.1)	
N2	158 (16.2)	41 (7.4)	117 (27.6)	
M stage (Yes)	95 (9.7)	6 (1.1)	89 (21.0)	<0.001
TNM stage (III-IV)	479 (49.1)	199 (36.1)	280 (66.0)	<0.001
Perineural invasion (Yes)	88 (9.0)	32 (5.8)	56 (13.2)	<0.001
Vascular invasion (Yes)	145 (14.9)	50 (9.1)	95 (22.4)	<0.001
Macroscopic type				
Protrude type	243 (24.9)	156 (28.3)	87 (20.5)	0.018

(Continued)

TABLE 1 Continued

Clinicopathological characteristics	Overall (n = 975)	Surviving patients (n = 551)	Deceased patients (n = 424)	p value
Infiltrating type	89 (9.1)	46 (8.3)	43 (10.1)	
Ulcerative type	643 (65.9)	349 (63.3)	294 (69.3)	
Differentiation (Poor)	116 (11.9)	53 (9.6)	63 (14.9)	0.016
Tumor location (Rectal)	476 (48.8)	262 (47.5)	214 (50.5)	0.401
Tumor size (median [IQR])	4.50 (3.50, 6.00)	4.50 (3.50, 6.00)	5.00 (4.00, 6.00)	0.009
CEA (High)	411 (42.2)	187 (33.9)	224 (52.8)	<0.001
Creatinine	0.87 (0.74, 1.01)	0.87 (0.75, 1.01)	0.86 (0.71, 1.00)	0.328
Cystatin C	0.92 (0.80, 1.06)	0.91 (0.80, 1.03)	0.96 (0.82, 1.10)	0.002
Creatinine/cystatin C ratios	93.09(80.89,106.90)	94.39(82.32,110.44)	91.20(79.17,101.99)	<0.001
Radiotherapy (Yes)	65 (6.7)	40 (7.3)	25 (5.9)	0.474
Chemotherapy (Yes)	496 (50.9)	272 (49.4)	224 (52.8)	0.313
HOS (median [IQR])	12.00(10.00,14.00)	11.00 (9.00, 14.00)	12.00 (10.75, 15.00)	<0.001
Hospitalization cost (median [IQR])	49325.05(44483.04, 55547.88)	48503.38(44304.62, 54646.96)	50845.43(44607.71, 56887.80)	0.008

CRC, colorectal cancer; BMI, body mass index; CCR, creatinine/cystatin C ratio.

(Figure S1). There were 734 CRC patients with a low creatinine/cystatin C ratio (<106.75) and 241 CRC patients with a high creatinine/cystatin C ratio (≥106.75). The median follow-up time of all patients was 72.8 months (38.9-88.4 months). A high creatinine/cystatin C ratio is significantly correlated with male gender, advanced age, low BMI, and prolonged hospital stay (Table 2).

Kaplan-Meier survival curve of creatinine/cystatin C ratio for PFS

During follow-up, a total of 282 patients (28.9%) developed recurrence and metastasis, including 227 patients in the low creatinine/cystatin C ratio group (30.9%) and 55 patients in the

TABLE 2 The relationships between the CCR and clinicopathological factors of CRC patients.

Clinicopathological characteristics	CCR		p value
	Low (n = 734)	High (n = 241)	
Sex(Man)	400 (54.5)	207 (85.9)	<0.001
Age (mean (SD))	58.97 (13.25)	53.02 (11.74)	<0.001
BMI (median [IQR])	21.67 (19.60, 24.00)	22.32 (20.32, 24.56)	0.006
Hypertension (Yes)	114 (15.5)	33 (13.7)	0.556
Diabetes (Yes)	51 (6.9)	10 (4.1)	0.160
T stage (T3-4)	554 (75.5)	181 (75.1)	0.976
N stage			0.134
N0	402 (54.8)	127 (52.7)	
N1	206 (28.1)	82 (34.0)	
N2	126 (17.2)	32 (13.3)	
M stage (Yes)	74 (10.1)	21 (8.7)	0.62
TNM stage (III-IV)	358 (48.8)	121 (50.2)	0.755
Perineural invasion (Yes)	72 (9.8)	16 (6.6)	0.174
Vascular invasion (Yes)	106 (14.4)	39 (16.2)	0.579
Macroscopic type			0.874

(Continued)

TABLE 2 Continued

Clinicopathological characteristics	CCR		p value
	Low (n = 734)	High (n = 241)	
Protrude type	182 (24.8)	61 (25.3)	
Infiltrating type	69 (9.4)	20 (8.3)	
Ulcerative type	483 (65.8)	160 (66.4)	
Differentiation (Poor)	93 (12.7)	23 (9.5)	0.236
Tumor location (Rectal)	364 (49.6)	112 (46.5)	0.444
Tumor size (median [IQR])	4.50 (3.50, 6.00)	4.50 (4.00, 6.00)	0.832
CEA (High)	318 (43.3)	93 (38.6)	0.224
Creatinine	0.81 (0.69, 0.94)	1.01 (0.89, 1.12)	<0.001
Cystatin C	0.96 (0.84, 1.10)	0.83 (0.75, 0.94)	<0.001
Creatinine/cystatin C ratios	87.31 (76.81, 95.49)	117.43 (111.88, 125.69)	<0.001
Death (Yes)	349 (47.5)	75 (31.1)	<0.001
Hospital stay (median [IQR])	12.00 (10.00, 14.00)	11.00 (9.00, 14.00)	0.006
Hospitalization cost (median [IQR])	49558.86 (44186.35, 55793.93)	48438.73 (44629.76, 54517.00)	0.260

CRC, colorectal cancer; BMI, body mass index; CCR, creatinine/cystatin C ratio.

high CCR group (22.8%). The 5-year RFS of patients in the low-creatinine/cystatin C ratio group was significantly lower than that in the high-creatinine/cystatin C ratio group (50.8% vs. 63.9%, p = 0.002) (Figure 1A). For stage I-II, PFS in patients with low creatinine/cystatin C ratio was significantly lower than that in patients with high creatinine/cystatin C ratio (66.0% vs 77.5%, p=0.041) (Figure 2A). For stage III-IV, we found that the creatinine/cystatin C ratio also significantly stratified the prognosis of CRC patients (34.9% vs 50.4%, p=0.006) (Figure 2C). In the normal CEA subgroup, we found that patients with low creatinine/cystatin C ratio had significantly lower 5-year PFS than those with high creatinine/cystatin C ratio (Figure S2A). However, no significant difference was observed in the high CEA subgroup (Figure S2C). The subgroup based on tumor location showed that the creatinine/cystatin C ratio could effectively stratify the prognosis of patients with rectal cancer (Figure S3A). Although the prognosis of rectal cancer with low creatinine/cystatin C ratio was worse than that of rectal cancer with high CCR, there was no significant difference (Figure S3C).

Kaplan-Meier survival curve of creatinine/cystatin C ratio for OS

During the follow-up period, a total of 424 patients (43.5%) died, including 349 patients in the low creatinine/cystatin C ratio group (47.5%) and 75 patients in the low creatinine/cystatin C ratio group (31.1%). Kaplan-Meier survival curve showed that patients with low creatinine/cystatin C ratio had significantly lower OS than that with high creatinine/cystatin C ratio (52.5% vs. 68.9%, p < 0.001) (Figure 1B). In the TNM stage subgroup analysis, we found that CCR can effectively stratify the prognosis of CRC patients with stage I-II (67.6% vs 81.7%, p=0.010) (Figure 2B) and stage III-IV (36.6% vs 56.2%, p=0.010) (Figure 2D). Likewise, the creatinine/

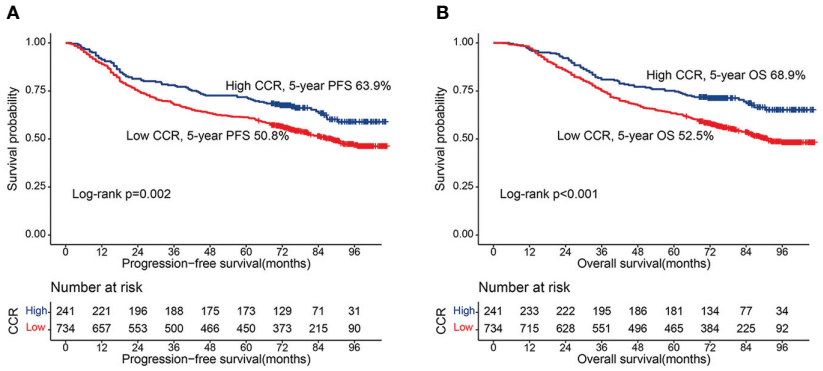

FIGURE 1
Kaplan-Meier curve of CCR in CRC patients. **(A)** Progression-free survival; **(B)** Overall survival.

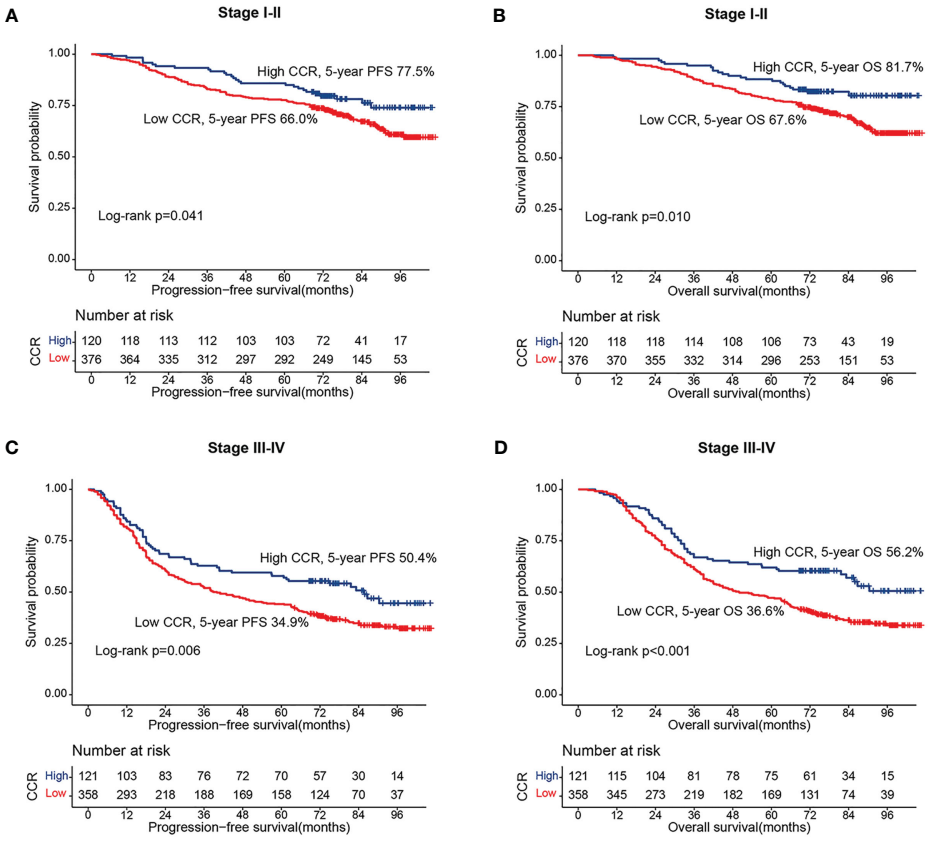

FIGURE 2
Stratified survival analysis of sarcopenia based on different TNM stage. **(A)** Progression-free survival of stage I-II; **(B)** Progression-free survival of stage III-IV; **(C)** Overall survival of stage I-II; **(D)** Overall survival of stage III-IV.

cystatin C ratio was able to significantly stratify the prognosis of patients with normal CEA (59.4% vs 79.1%, p<0.001) (Figure S2B), but not high CEA (Figure S2D). It is worth noting that the creatinine/cystatin C ratio can perform good prognostic differentiation on rectal cancer (50.8% vs 68.8%, p=0.003) and colon cancer (54.1% vs 69.0%, p=0.009) (Figure S3B, D).

Multivariate analysis of predictors for PFS

RCS showed that with the increase of creatinine/cystatin C ratio, The PFS of CRC patients gradually increased. After correcting for confounding factors, there was still a negative linear relationship between creatinine/cystatin C ratio and adverse PFS of CRC patients (Figure 3). In univariate analysis, PFS was affected by the following clinical characteristics: age (p=0.005), BMI, T stage (p<0.001), N stage (p<0.001), M stage (p<0.001), perineural invasion (p<0.001), vascular invasion (p<0.001), pathological type, differentiation (p=0.011), CEA (p<0.001) and creatinine/cystatin C ratio (p=0.002). Subsequent multivariate analysis of the 11 significant factors in the univariate analysis showed that the independent prognostic factors affecting PFS in CRC patients were age (HR=1.318, 95%CI=1.076–1.615, p=0.008), creatinine/cystatin C ratio (HR=1.286, 95%CI = 1.007–1.642, p=0.044), T stage (HR=1.559, 95%CI =1.173–2.073, p=0.002), N stage (p<0.001), M stage (HR=3.628, 95%CI=2.814–4.677, p<0.001) and CEA (HR=1.286, 95%CI=1.007–1.642, p=0.003) (Table 3). We performed a multivariate subgroup analysis based on various clinical features. The results showed that the low creatinine/cystatin C ratio was a risk factor for PFS in most of the subgroups (Figure S4A).

Multivariate analysis of predictors for OS

In univariate Cox proportional hazard regression models, patients with low creatinine/cystatin C ratio had 1.410 times the risk of adverse OS compared with patients with high creatinine/cystatin C ratio (HR = 1.421, 95%CI =1.168 – 1.730, p<0.001). After adjusting for confounding factors, advanced age (HR=1.013, 95% CI=1.005–1.021, p=0.001), low creatinine/cystatin C ratio (HR=1.410, 95%CI=1.087–1.829, p=0.010), advanced T stage (HR=1.578, 95%CI=1.175–2.120, p=0.002), advanced N stage (p<0.001), advanced M stage (HR=3.879, 95%CI=3.001–5.013, p<0.001) and high CEA (HR=1.333, 95%CI=1.084–1.641, p=0.007) were independently associated with poor OS in CRC patients (Table 4). Multivariate subgroup analysis showed that a low creatinine/cystatin C ratio was a risk factor for OS in most subgroups of CRC patients (Figure S4B).

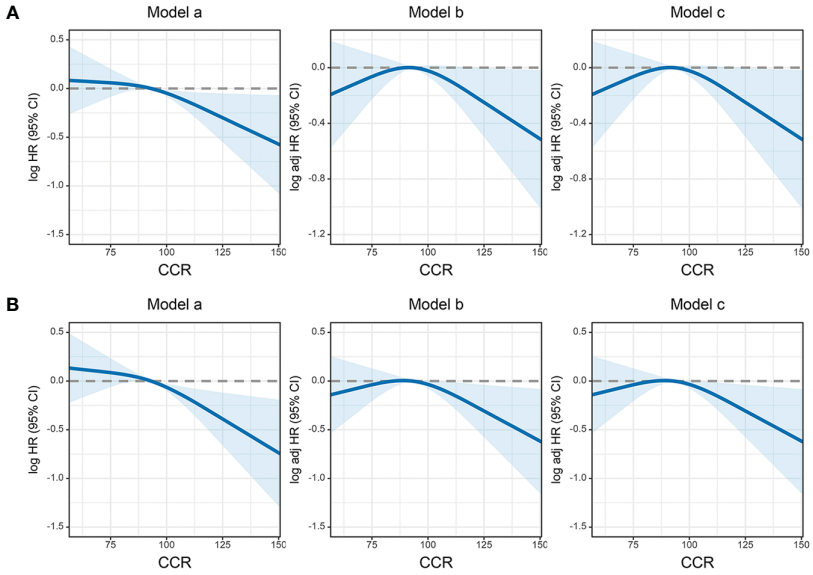

FIGURE 3
The association between CIPI and survival in patients with colorectal cancer. **(A)** Progression-free survival; **(B)** Overall survival. Model a: No adjusted. Model b: Adjusted for gender, age, and BMI. Model c: Adjusted for gender, age, BMI, hypertension, diabetes, T stage, N stage, M stage, tumor location, tumor size, perineural invasion, vascular invasion, macroscopic type, differentiation, radiotherapy, chemotherapy.

Construction of prognosis prediction model

To evaluate the prognosis of CRC patients comprehensively, we have built prognostic nomograms for predicting the prognosis of 1-5 years of CRC patients. Based on all independent indicators in the multivariate analysis of PFS, we constructed a PFS nomogram, including age, T stage, N stage, M stage, CEA levels, and creatinine/cystatin C ratio (Figure 4A). The higher the nomogram score, the worse the clinical prognosis of CRC patients. The C-index of the PFS nomogram was 0.719 (95%CI: 0.695-0.743). The 3 - and 5-year calibration curves showed good agreement between the predicted values of the nomogram and observed values (Figures S5A, B). Similarly, we included significant variables in the multivariate analysis of OS to construct an OS nomogram (including age, T stage, N stage, M stage, vascular invasion, CEA level, and creatinine/cystatin C ratio) (Figure 4B). The C-index of the OS nomogram was 0.727 (95%CI: 0.703-0.752). The 3 -year and 5 -year calibration curves demonstrated the best agreement between the survival probability predicted by the OS nomogram and the actual observed values (Figures S5C, D). Subsequently, we compared the constructed nomograms with the traditional TNM staging through the ROC curve. Compared with TNM staging, the constructed nomograms had better resolution and accuracy in predicting 3 - and 5-year PFS (3-year AUC: 0.773 vs 0.734; 5-year AUC: 0.767 vs 0.720) (Figures S6A, B). Similarly, the constructed nomograms were better at predicting the performance of the 3 - and 5-year OS than the TNM staging (3-year AUC: 0.782 vs 0.742; 5-year AUC: 0.772 vs 0.718) (Figures S6C, D).

Internal queue validation

We randomly divide all patients into two cohorts at a ratio of 7: 3: validation cohort A (684 cases) and validation cohort B (291 cases). Table S1 showed that there was no statistical significance in clinicopathological characteristics between the validation cohort A group and validation cohort B group. In the validation cohort, A, patients in the low-creatinine/cystatin C ratio group had significantly lower PFS (51.1% vs. 62.3%, p = 0.036) (Figure 5A) and OS (53.0% vs. 67.1%, p = 0.007) (Figure 5B) than those in the high-creatinine/cystatin C ratio group. In validation cohort B, the creatinine/cystatin C ratio was still able to effectively stratify the prognosis of CRC patients (PFS, 50.2% vs. 67.6%, p = 0.012; OS, 51.2% vs. 73.0%, p = 0.002) (Figures 5C, D). The C-index of PFS and OS nomograms was 0.715 (95%CI: 0.687-0.743) and 0.726 (95%CI: 0.698-0.754) at validation cohort A, respectively. In validation cohort B, The C-index of PFS and OS nomograms was 0.742 (95%CI: 0.700-0.785) and 0.740 (95%CI: 0.696-0.785), respectively. In addition, calibration curves of 3- and 5-year PFS/OS in validation cohort A (Figures S7A, B) and validation cohort B (Figures S7C, D) showed good agreement between predicted and observed values.

Discussion

In this study, we demonstrated for the first time that the creatinine/cystatin C ratio is an important predictor of PFS and OS in CRC patients. With the increase of creatine-cystatin C ratio, the HRS of

TABLE 3 Univariate and multivariate Cox regression analysis of clinicopathological characteristics associated with progression-free survival in CRC patients.

Clinicopathological characteristics	Progression-free survival			
	Univariate analysis		Multivariate analysis	
	HR (95%CI)	P value	HR (95%CI)	P value
Age	1.315 (1.085-1.594)	0.005	1.318 (1.076 - 1.615)	0.008
BMI				
Low	Ref.		Ref.	
Normal	0.843 (0.651-1.091)	0.194	0.904 (0.695 - 1.175)	0.450
High	0.673 (0.498-0.909)	0.010	0.799 (0.588 - 1.084)	0.149
T stage (T3/4)	2.48 (1.9-3.238)	<0.001	1.559 (1.173 - 2.073)	0.002
N stage				
N0	Ref.		Ref.	
N1	1.724 (1.385-2.145)	<0.001	1.457 (1.16 - 1.83)	0.001
N2	3.754 (2.978-4.733)	<0.001	2.692 (2.096 - 3.459)	<0.001
M stage	5.693 (4.483-7.228)	<0.001	3.628 (2.814 - 4.677)	<0.001
Perineural invasion (Positive)	1.788 (1.36-2.352)	<0.001	1.094 (0.806 - 1.483)	0.565
Vascular invasion (Positive)	1.937 (1.547-2.427)	<0.001	1.284 (0.991 - 1.662)	0.058
Pathological type				
Protrude type	Ref.		Ref.	
Infiltrating type	1.447 (1.017-2.061)	0.04	1.196 (0.835 - 1.713)	0.328
Ulcerative type	1.29 (1.023-1.626)	0.032	1.077 (0.85 - 1.365)	0.537
Differentiation (High-medium)	0.707 (0.541-0.923)	0.011	0.901 (0.681 - 1.193)	0.468
CEA (≥5ng/ml)	1.919 (1.593-2.31)	<0.001	1.358 (1.111 - 1.66)	0.003
CCR (Low)	1.456 (1.152-1.84)	0.002	1.286 (1.007 - 1.642)	0.044

CRC, colorectal cancer; BMI, body mass index; CCR, creatinine/cystatin C ratio.

mortality of CRC patients gradually decreases. The creatinine/cystatin C ratio can also be used as an effective auxiliary tool for pathological staging to further distinguish the prognosis of CRC patients with the same pathological stage. We also found that CCR could further stratify the prognosis of CRC patients with normal CEA, but was not suitable for patients with high CEA. In addition, we constructed CCR-based prognostic nomograms to predict 1-5year PFS/OS in CRC patients and validated the good predictive performance of these nomograms through the random internal cohorts.

At present, the relationship between the creatinine/cystatin C ratio and the prognosis of patients with cancer has attracted more and more attention. Jung et al. (16) found that the creatinine-cystatin C ratio was significantly associated with reduced 6-month mortality of patients with cancer. Ding et al. (17) also found that the creatinine/cystatin C ratio was independently correlated with sarcopenia and relapse-free survival in patients with gastrointestinal stromal tumors. The study of Zheng et al. (11) also showed that creatinine/cystatin C ratio can be used to identify sarcopenia and is a useful prognostic factor for postoperative complications and long-term survival in patients with esophageal cancer. A study by Chen et al. involving 664 non-small cell lung cancer patients found that creatinine/cystatin C ratio was associated with mortality in women, but not in men (18). The results of this study showed that CRC patients in the high creatinine/cystatin C ratio had significantly higher RFS/OS than those in the low serum creatinine/cystatin C ratio group. Multivariate analysis showed that CRC patients with low creatinine/cystatin C ratio had 28.6% and 41.0% higher adverse PFS and OS than CRC patients with high creatinine/cystatin C ratio, respectively.

Pathological stages and serum CEA levels are important factors in assessing the prognosis of CRC patients (5). However, even with the same pathological stage, the prognosis of patients varies greatly. In this study, we found that the creatinine/cystatin C ratio can effectively stratify the prognosis of CRC patients with the same level of pathological staging, suggesting that the creatinine/cystatin C ratio can be a useful supplement in predicting the prognosis of CRC patients. Studies have shown that serum CEA is not specific in CRC patients, and more than 50%of CRC patients have negative serum CEA (19, 20). We found that the creatinine/cystatin C ratio can be used as an effective prognostic stratification factor for CRC patients with normal CEA levels, suggesting that it can be used as a further

TABLE 4 Univariate and multivariate Cox regression analysis of clinicopathological characteristics associated with overall survival in CRC patients.

Clinicopathological characteristics	Overall survival			
	Univariate analysis		Multivariate analysis	
	HR (95%CI)	P value	HR (95%CI)	P value
Age	1.421 (1.168-1.73)	<0.001	1.013 (1.005 - 1.021)	0.001
BMI				
Low	Ref.			
Normal	0.846 (0.648-1.102)	0.215	0.920 (0.702 - 1.207)	0.548
High	0.661 (0.484-0.902)	0.009	0.787 (0.573 - 1.079)	0.137
T stage (T3/4)	2.52 (1.91-3.326)	<0.001	1.578 (1.175 - 2.120)	0.002
N stage				
N0	Ref.			
N1	1.7 (1.357-2.129)	<0.001	1.451 (1.146 - 1.837)	0.002
N2	3.77 (2.974-4.779)	<0.001	2.633 (2.034 - 3.409)	<0.001
M stage	6.096 (4.792-7.755)	<0.001	3.879 (3.001 - 5.013)	<0.001
Perineural invasion (Positive)	1.782 (1.345-2.36)	<0.001	1.052 (0.768 - 1.442)	0.750
Vascular invasion (Positive)	2.019 (1.606-2.538)	<0.001	1.335 (1.024 - 1.74)	0.033
Pathological type				
Protrude type				
Infiltrating type	1.425 (0.989-2.053)	0.058	1.208 (0.831 - 1.756)	0.322
Ulcerative type	1.295 (1.02-1.646)	0.034	1.118 (0.873 - 1.43)	0.377
Differentiation (High-medium)	0.648 (0.496-0.847)	0.001	0.802 (0.602 - 1.07)	0.134
Size (≥5cm)	1.275 (1.053-1.543)	0.013	1.061 (0.869 - 1.296)	0.563
CEA (≥5ng/ml)	1.896 (1.566-2.295)	<0.001	1.333 (1.084 - 1.641)	0.007
CCR (Low)	1.646 (1.283-2.113)	<0.001	1.410 (1.087 - 1.829)	0.010

CRC, colorectal cancer; BMI, body mass index; CCR, creatinine/cystatin C ratio.

prognostic stratification factor for CEA in the prognosis prediction of CRC patients. Furthermore, the high creatinine/cystatin C ratio was a prognostic factor for poor PFS/OS in patients with colon cancer. However, the creatinine/cystatin C ratio is a useful prognostic factor in predicting OS in rectal cancer patients, but not PFS. Overall, the creatinine/cystatin C ratio can be considered a universally applicable, readily available, and effective method for predicting the risk of poor outcomes in CRC patients.

One possible explanation for the association between the creatinine-cystatin C ratio and the outcome of CRC patients is that the creatinine-cystatin C ratio represents muscle mass, which is a well-known risk factor for the outcome of CRC patients (21). A recent study showed that the creatinine/cystatin C ratio was significantly correlated with CT and BIA in assessing muscle mass, and could be conveniently used as a reliable biomarker for muscle in patients with cancer (22). Similarly, a study by Tlemsani et al. also showed that the creatinine/cystatin C ratio is a useful and simple biomarker for predicting sarcopenia in patients with cancer. In addition, the index also appears to be a strong biomarker for the diagnosis of sarcopenia in overweight and obese cancer patients (11). These studies further support this explanation. Other research suggested that the creatinine/cystatin C ratio may also be a sign of systemic inflammation (16, 23). Systemic inflammation is the most representative interaction between tumor and host, and increased inflammation load is an important factor affecting the prognosis of cancer patients (7). Serum creatinine levels were lower in patients with high white blood cell counts, while elevated levels of cystatin C were observed in chronic inflammatory states. The decrease in the creatinine/cystatin C ratio reflects the accumulation of inflammatory load in the body. Therefore, the creatinine/cystatin C ratio may be a promising prognostic biomarker in CRC patients.

Further, we constructed creatinine/cystatin C ratio-based prognostic nomograms to predict the 1–5-year prognosis of CRC patients. These nomograms have good predictive performance, with C-index can reach above 0.7. Subsequently, we demonstrated that these nomograms had good application prospects through validation cohorts. Compared to traditional pathological stages, these nomograms have better prognostic prediction efficiency. In summary, these nomograms have good prognostic efficacy, which can help to provide individualized recommendations for prognostic

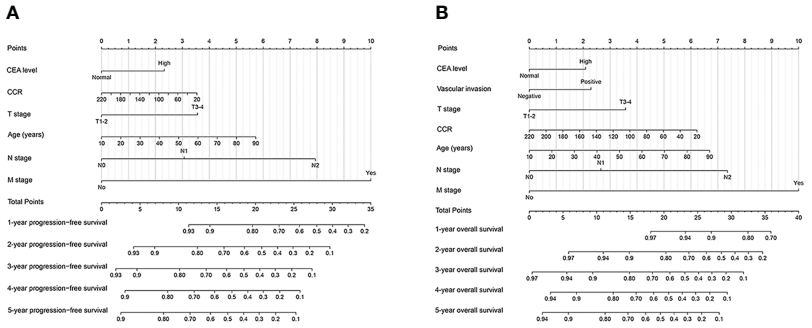

FIGURE 4
Construction prognostic nomograms in CRC patients. **(A)** The Progression-free survival nomogram; **(B)** The overall survival nomogram.

prediction, efficacy evaluation, and treatment formulation of CRC patients.

As far as we know, this study is the first to confirm that the creatinine/cystatin C ratio is an independent predictor of PFS and OS in CRC patients. Creatinine/cystatin C ratio also can help pathological staging and tumor markers to stratify the prognosis of CRC patients in more detail. In addition, we further constructed the prognostic nomograms based on the creatinine/cystatin C ratio, which can be more personalized and convenient to be used in clinical practice. However, there are still some limitations worth noting. This is a single-center retrospective study, with problems such as small sample size and patient selection bias. Secondly, this study lacks data to evaluate sarcopenias, such as CT, DXA, and BIA, which further restricts the explanation of the association between creatinine/cystatin C ratio and muscle mass. As patients can experience kidney damage due to surgery, radiation therapy, and chemotherapy, resulting in fluctuations in creatinine and cystatin C levels, peripheral venous blood samples that include Creatinine and Cystatin C were collected from all patients after an overnight fast during the week before surgery in this study. However, as this study only collected single-time serum data, we

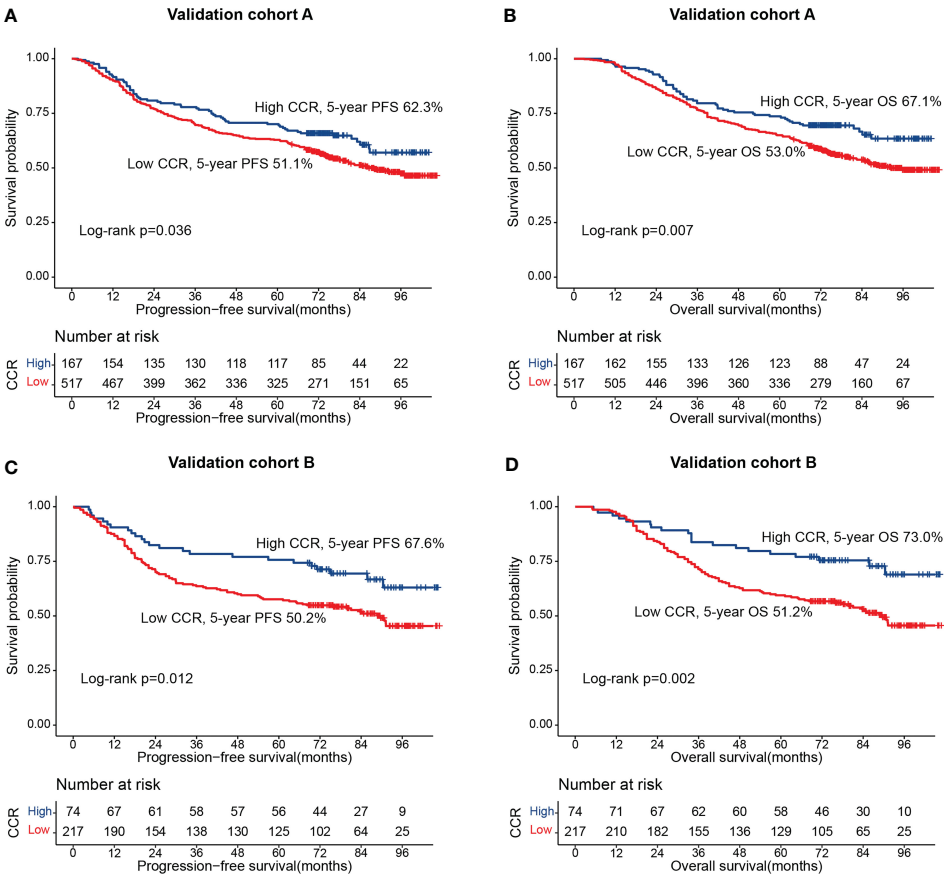

FIGURE 5
The association between CCR and survival in patients with colorectal cancer in validation cohorts. **(A)** Progression-free survival at validation cohort A; **(B)** Overall survival at validation cohort A; **(C)** Progression-free survival at validation cohort B; **(D)** Overall survival at validation cohort B.

were unable to explore the impact of the trajectory changes of Creatinine/cystatin C ratio on prognosis. Finally, this study lacks an independent validation cohort, which is an additional limitation. Therefore, further prospective studies with multi-center and large sample sizes are needed.

Conclusion

Creatinine/cystatin C ratio may be an effective prognostic marker for predicting PFS and OS in CRC patients and can help pathological staging and tumor markers to perform more detailed prognostic stratification in CRC patients. The creatinine/cystatin C ratio-based nomograms have good prediction accuracy and can individually help identify high-risk patients who have an adverse prognosis.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding authors.

Ethics statement

The studies involving human participants were reviewed and approved by the Institutional Review Board of the First Affiliated Hospital, Guangxi Medical University. The patients/participants provided their written informed consent to participate in this study.

Author contributions

JG, SG, and HX carried out the design of this study, analyses of statistics and draft the manuscript. HX, LW, ML, YL, QW, and ST carried out collection of the statistics and prepared the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

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.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2023.1155520/full#supplementary-material

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Eicosapentaenoic acid and branched-chain amino acids fortified complete nutrition drink improved muscle strength in older individuals with inadequate protein intake

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Background: Elevated inflammation and negative nutritional balance contribute to sarcopenia, a progressive loss of muscle mass, strength, and function. This study investigated the effect of energy supplementation and the combination of anti-inflammatory factor (eicosapentaenoic acid; EPA) and muscle-synthesis promotor (branched-chain amino acids; BCAA) on body composition, muscle, and inflammatory biomarkers in elderly with inadequate protein intake.

Methods: A randomized blinded placebo-controlled trial was conducted on 84 elderly with inadequate protein intake. The participants were randomly assigned into four groups receiving a complete nutrition drink; (1) control formula, (2) fortified with 2.2 g EPA, (3) with 2.2 g EPA and 5 g BCAA (2:1:1 of Leu: Ile: Val), and (4) with 2.2 g EPA plus 5g BCAA (4:1:1 of Leu: Ile: Val). Each subject consumed two sachets of the drink to gain 500 kcal/day and performed arm muscle exercises for 3 weeks. Body compositions and handgrip strength were measured using BIA and a dynamometer, respectively. Plasma EPA and BCAA levels were determined using LC-MS/MS to ensure compliance. Muscle protein biomarkers including histidine, β-alanine, and carnosine were measured using LC-MS/MS. Serum inflammatory (IL-6) and anti-inflammatory cytokines (IL-10) were measured by using ELISA.

Results: No symptoms and signs of adverse events were observed. The right arm muscle mass and handgrip strength were significantly increased after consuming a complete nutrition drink fortified with EPA + BCAA 2:1:1 and 4:1:1 of Leu: Ile: Val ($p < 0.05$ and $p < 0.01$, respectively. Consistently, consuming such combinatory formula non-significantly elevated carnosine with reduced histidine, and increased IL-10 with decreased IL-6. All relevant intervention groups showed a significant increase in plasma levels of BCAA and EPA.

Conclusion: Consuming a complete nutrition drink fortified with 2.2g EPA and 5g BCAA 2:1:1 or 4:1:1 of Leu: Ile: Val for 3 weeks may increase right arm muscle mass and strength in elderly with inadequate protein intake. The tendency of increased dipeptide (carnosine)/decreased free amino acid (histidine) suggests a shift toward

muscle protein synthesis. The trend of decreased inflammatory/increased anti-inflammatory cytokines suggests an anti-inflammatory effect. Future long-term studies are warranted to confirm the combinatory effect of BCAA and EPA in the prevention of sarcopenia.

Clinical trial registration: Thailand Clinical Trial Registry No. TCTR20230116005.

KEYWORDS

EPA, BCAA, sarcopenia, muscle, complete nutrition drink, elderly, protein, randomized controlled trial

Introduction

Older populations are at risk of sarcopenia, i.e., an age-related, involuntary loss of skeletal muscle mass, and strength (1). Compelling evidence suggests that skeletal muscle mass and skeletal muscle strength drop linearly, with up to 50% loss of muscle mass by the age of 80 and above (1). Protein-energy malnutrition is associated with an increased risk of sarcopenia (2, 3). Our and other previous studies showed that inadequate protein intake is associated with low muscle mass index in elderly people (4, 5). Worsen the problem, aging leads to the deterioration of organs involved in nutrition intake and metabolism; thus, the risk of malnutrition-related sarcopenia is high in the elderly population (6–8). Importantly, sarcopenia can reduce the quality of life, lessen tolerance to illness, and increase the risk of mortality (9, 10). Nevertheless, no standard interventions for sarcopenia had been accepted, and neither does conventional nutritional support can exclusively resolve sarcopenia (11). The European Society for Clinical Nutrition and Metabolism (ESPEN) recommends using a multimodal approach in the management of sarcopenia (12). Convincing pieces of evidence suggested that the mechanism of sarcopenia involves systemic inflammation leading to increased degradation of protein and lipid from muscle and adipose tissue storage (13).

Previous studies demonstrated the potential benefit of eicosapentaenoic acid (EPA) to alleviate sarcopenia in the elderly, showing improvement in body weight, muscle mass, and function (14). A possible mechanism of EPA likely involves anti-inflammation and downregulation of proteasome expression (15). The anti-inflammatory mechanisms of EPA include protection against the disruption of the cell membrane and inactivation of transcription factor NFκB leading to the suppression of gene transcription for pro-inflammatory cytokines such as IL-1, IL-6, IL-8, and IL-12 (16, 17). Furthermore, EPA is also an inhibitor of platelet-activating factor (PAF) (18), which is a potent lipid inflammatory mediator (19) negatively affecting whole-body and skeletal muscle protein synthesis (20) Moreover, a plasma lipid profile of lower omega 6, which antagonize omega 3, has been associated with reduced PAF biosynthesis and/or increased catabolism (21). A dietary pattern rich in fish and other healthy food groups has been inversely associated with circulating PAF plasma levels (22). Interestingly, a recent study showed that the supplementation of an amino acid-rich diet after exercise could preserve lean body mass in older persons (23). Another study showed that a single dose of amino acid-rich complete nutrition drinks could stimulate the rate of muscle synthesis (24). The effect may be due to branched-chain amino acids (BCAAs) especially leucine which is critical protein synthesis for muscle (24). Leucine was shown to be the primary stimulator of muscle protein synthesis via the mammalian target of the rapamycin complex 1 (mTORC1) pathway (25). Therefore, the elevation of plasma leucine concentration by providing leucine-enriched nutrition supplements with an adequate energy drink can resume muscle protein synthesis, while appropriate menu changes can increase leucine dietary intake in patients (26). Nevertheless, it is still unclear if a combination between EPA and BCAA would be effective in improving muscle health and reducing the risk of sarcopenia in the elderly at risk. Recently, the EPA–BCAA-fortified nutrition drink has been developed and sterilized with a retort. It passed the microbial safety test and had over 80% sensory acceptance (27). Thus, this study aimed to investigate the effect of energy supplementation and the combination of anti-inflammatory factor (eicosapentaenoic acid; EPA) and muscle-synthesis substrate (branched-chain amino acids; BCAA) on body composition, muscle degradation, and inflammatory biomarkers in elderly people with inadequate protein intake.

Materials and methods

Ethical aspects and setting

Data were collected at the Institute of Nutrition, Mahidol University, Thailand. The study protocol complied with the Declaration of Helsinki and the International Conference on Harmonization Guidelines for Good Clinical Practice (ICH-GCP), and was approved by the Ethical Committees for Mahidol University Central Institutional Review Board (MU-CIRB) with protocol No. 2021/289.0106 Approval No. 2021/169.1507. The protocol namely "Novel approach to prevent sarcopenia using a combination of eicosapentaenoic acid (EPA) and branch-chained amino acids (BCAAs): a pilot study in elderly people with inadequate protein intake" was registered in the Thai Clinical Trials Registry (TCTR) with protocol No. TCTR20230116005. It can be accessed at https://www.thaiclinicaltrials.org/#. All participants provided written informed consent before enrollment.

Participants

This study recruited 60–90-year-old healthy people who had no systemic diseases or had stable and controlled diseases, e.g., diabetes, hypertension, or dyslipidemia. Elderly with controlled diabetes must have HbA1C < 9%, FBS <180 mg/dL, and no drug adjustment throughout the period of data collection. Subjects should have normal liver and renal function based on ALT < 60 IU/L and eGFR ≥ 60 ml/min. All of the participants were evaluated for the risk of malnutrition leading to sarcopenia. They noticeably had inadequate protein intake; consuming <0.5 g/ kg body weight during the past 7 days or <0.7 g/ kg body weight during the past 14 days. The criteria for inadequate protein intake were set up according to the ESPEN guidelines (12). Inadequate protein intake was defined as consuming <50% of the nutritional goal within 7 days or experiencing lower intake than 70% of the individual target constantly, as assessed via dietary recall at screening. The goal for protein intake was 1 g/ kg body weight per day (12). The participants were excluded if they had communication problems, cancer, liver disease, renal disease at stages 3 to 5, Alzheimer's disease, malabsorption such as short bowel syndrome, alcoholism, inflammatory bowel disease (IBD), and pancreatitis, allergic to fish, soybean, peanut, and milk, blood coagulation problems (e.g., taking anti-coagulants (e.g., warfarin), or having some diseases such as thrombocytopenia or leukemia). The participants were acknowledged of the purpose and protocol of the research and given information documents. Then, the volunteers signed a written consent to participate in this research project.

Sample size and power

The sample size was calculated by using G power V.3.1.9.4. Power of 0.8, theoretical large effect size (0.4) for one-way ANOVA of four groups, and a level of significance (α) of 0.05, was used for calculation. The non-centrality parameter combination (NPC) is 12.16 with a critical F-value of 2.73 which contributed to the total of 76 subjects (19 in each group). Adding the anticipated 10% drop-out, the total sample size was 84 participants (21 in each group).

Study design blinding, random allocation, and procedures

The blinded randomized control procedure was achieved. All participants were blinded from the intervention assignment by labeling the package of complete nutrition drinks with A, B, C, and D. The random group assigner, data collector, laboratory analyzer, and statistical analyzer were different persons. As shown in Supplementary Figure S1, the 84 participants were randomly assigned into 4 groups equally with minimization using matched age, sex, body mass index, and amount of protein and energy intakes. The four groups received different formulas of complete nutrition drinks: (1) control formula, (2) fortified with 2.2 g EPA, (3) fortified with 2.2 g EPA and 5 g BCAA (2:1:1 of Leu: Ile: Val), and (4) fortified with 2.2 g EPA plus 5g BCAA (4:1:1 of Leu: Ile: Val). Each participant was asked to consume two sachets of the complete nutrition drinks provided 500 kcal/day and 25 g protein to ensure decent energy and protein for daily requirements. Clinical and laboratory outcome parameters were measured at 0 and 3 weeks after interventions. Each participant was asked to do overnight fasting before 10 ml of blood was collected during each visit. The blood was divided into several portions; plasma for measuring complete blood count (CBC), glucose, cholesterol, liver, and kidney function markers, muscle synthesis/ degradation markers, EPA, BCAA, carnosine histidine, and beta-alanine, and serum for measuring cytokines.

Interventions

The complete nutrition drinks were produced as described (27). Briefly, the placebo control formula liquid was made from Blendera-MF powder (Thai Otsuka Pharmaceutical Co., Ltd., Samut Sakhon, Thailand). The fortified formulas, fish oil containing EPA (PronovaPure®, BASF Pharma, Florham Park, NJ, USA) and/ or BCAAs (leucine, isoleucine, and valine from Ajinomoto Co., Ltd. (Thailand), were added and mixed into the control formula liquid. Each formula liquid was filled in the retort pouch for 250 mL per sachet. The pouches were sterilized by retort at 116°C for 25 min. This condition was selected to achieve a lethality value (F0) and ensure sterility as described (27). The products passed microbial safety for low-acid canned food according to Codex standards. Supplementary Table S1 describes the ingredients of all formulas. Nutrition values are as described (27). Our previous study showed that retort sterilization resulted in a 30% reduction in EPA content with no effects on the amount of BCAA (27). Thus, in this study, we increased the amount of EPA used as a raw material by 30%, resulting in a product that, after sterilization, comprises 1.1 g of EPA and 2.5 g BCAA per 250 mL sachet. Consuming two sachets per day will provide 2.2 g of EPA and 5 g of BCAA as designed.

Consented participants who met inclusion criteria and agreed to join the study were provided with complete nutrition drinks according to the random assignment. If the participants also had inadequate energy intake from regular food, they were instructed to consume the drink as a supplement in addition to regular meals. However, if the participants already had adequate energy intake from regular food, they were asked to consume the drink as a replacement for breakfast. All participants continued to consume two 250 mL sachets of the assigned drinks daily for 3 weeks. Furthermore, all of them were instructed to consistently perform arm muscle exercises by squeezing the given resistance rubber handgrip ring (Decathlon®) twice daily for 10 min/ set. All participants were advised to maintain their dietary behavior throughout the study.

Monitoring

To monitor dietary behavior, all participants were asked to record in their subject diaries throughout the study. They filled up dietary records of their intakes every week. In each week

of recording, 2 weekdays and 1 weekend data were obtained. To monitor safety, all participants were asked to record any adverse symptoms associated with the supplementation in subject diaries. In addition, routine blood chemistry including complete blood count (CBC), glucose, lipid profile, and liver and kidney markers were measured at 0 and baseline and 0 and 3 weeks after interventions. On a follow-up visit, all subjects were also interviewed for adverse events.

Compliance

To ensure compliance in consuming the assigned formula, participants were asked to make daily records of consumption and return the empty sachets at the follow-up visits. Furthermore, plasma levels of EPA and BCAA were measured to ensure compliance (see next section for the laboratory methods).

Outcome measurement

Body composition and muscle strength

Body weight, muscle, and fat mass values were determined by bioelectrical impedance analysis (BIA) using the Tanita BC-545 body composition analyzer (Tanita Cooperation, Tokyo, Japan). Handgrip muscle strength of the right and left arms was determined by a hand-held dynamometer (28) using Jamar Plus + Digital 563213 (Lafayette Instrument Company, IN, USA).

Laboratory analyses

Plasma preparation for chemical evaluation via LC-MS/MS

The blood samples were collected. Blood samples were centrifuged at 1000 rpm at room temperature for 10 min. The upper layer of plasma was collected and stored at −20°C until analyses.

Analysis of EPA via LC-MS/MS

Plasma EPA levels were determined by using a method described previously (29). An aliquot of 100 μL of plasma was used to extract lipid with hexane/isopropanol. The extraction began with 3:2 volume by volume of hexane: isopropanol at 1:10 volume by volume of plasma to solvent. After mixing well with the vortex, the mixture was incubated at −20°C for 10 min, followed by centrifugation at 14,000 g at 4°C for 5 min. Then, the hexane extract layer was collected and dried to remove solvents using a SpeedVac concentrator (CentriVap Benchtop Vacuum Concentrators, Labconco, USA) at room temperature for 15 min. One milliliter of 80% methanol was added to the extracted plasma. Nine hundred microliters of the extract were alkaline hydrolyzed by adding 100 μL of 0.3 M KOH in 80% MeOH and incubating at 80°C for 30 min. After the sample was cooled down, 10 μL of formic acid was added to adjust the pH. One milliliter of hexane was added for the extraction of fatty acids and mixed for 5 min, followed by centrifugation at 1000 g. The hexane layer was collected and evaporated using a SpeedVac concentrator to remove the solvent. Then, the sample was reconstituted with 1 mL of 80% methanol and filtered through a 0.2 μm Nylon filter prior to liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis. For calibration curve generation, 0, 125, 250, 500, 750, and 1000 ng/mL of EPA in 80% methanol were used. An internal standard, EPA-d5 (MaxSpec Cayman) was added to all plasma samples and standard solutions equally at the beginning of sample preparation.

Liquid chromatography was completed with Ultimate 3000 Ultra High-performance liquid chromatography (UHPLC, Thermo Scientific, Waltham, MA, USA) well-assembled with a Hypersil GOLD™ C18 column (100 × 2.1 mm, particle size 1.9 μm) at 50°C. The prepared mobile phases were acetonitrile (mobile phase A) and 5 mM ammonium acetate in water (mobile phase B), respectively. The flow rate condition was established at 0.6 mL/min with 10 min period per injection. The mobile phase was regulated in a gradient form, with the percentage of mobile phase A being 0–6.5 min: 60% A, at 6.5–9 min: 98% A, and at 9–10 min: 60% A. Each single injection quantity was 5 μL. The retention times at 1.7 min were similar to EPA and EPA-d3. Mass spectrometry was connected to liquid chromatography with TSQ Quantis triple quadrupole mass spectrometer (Thermo Scientific, USA). The mass spectrometry setting was negative ion electrospray ionization (-ESI) with spraying voltage at 3500 V under the N2 sheath. Sheath and auxiliary gases were 30 and 15 arbitrary (Arb) units, respectively. The temperature of the ion transfer tube (ITT) was 325°C, while the vaporizing temperature was higher at 350°C. The selected reaction monitoring (SRM) mode for real-time analysis of multiple masses mass was executed for spectrometer analysis. The mass-to-charge ratio (*m/z*) of EPA precursor was 301. The quantified product mass of EPA was *m/z* 257. A collision energy of 12 V was used for the transition. The confirmed product mass for EPA was *m/z* 203.3 with a collision energy of 13 V for the transition. The mass-to-charge ratio (*m/z*) of EPA-d5 precursor and quantified product masses was 306.3. The confirmed product mass for EPA-d5 was *m/z* 262.3 with a collision energy of 12 V for the transition.

Analysis of plasma BCAA via LC-MS/MS

Plasma BCAA levels were determined by using a method described previously (30). An aliquot of 100 μL of plasma was mixed with 10 μL of 30% sulfosalicylic acid in an Eppendorf tube. The mixture was mixed well and kept under 4°C for 30 min (30). The mixture was separated via centrifugation at 12000 rpm for 5 min. A measure of 50 μL of supernatant was collected. The sample was filtered through a 0.2 μm Nylon filter and kept in a glass vial for further analysis. Standards of valine, isoleucine, and leucine used in this study are the metabolomics amino acid mixtures unlabeled standard (MSK-A2-US-1.2: Cambridge Isotope Laboratories, Inc., USA). Internal standards including 13C/ 15N-valine, 13C/15N-isoleucine, and 13C/15N-leucine were supplied by stable isotope-labeled metabolomics amino acid mixtures (MSK-A2-1.2: Cambridge Isotope Laboratories, Inc., USA).

Liquid chromatography was accomplished with Ultimate 3000 Ultra High-performance liquid chromatography (UHPLC, Thermo Scientific, Waltham, MA, USA) well-assembled with a Hypersil GOLD^{TM} C18 column (100 × 2.1 mm, particle size 1.9 μm) at 30°C. The mobile phase solution for chromatography was prepared with 95% of methanol: 5% of 20 mM Ammonium formate in water. The analytical solution had a flow rate of 0.3 mL/min and a run time of 5 min per injection. The individual volume was 5 μL per single injection. The retention times according to the protocol of mixed amino acids standard of valine, ^{13}C/ ^{15}N-valine, isoleucine, ^{13}C/ ^{15}N-isoleucine, leucine, and ^{13}C/ ^{15}N-leucine were 1.21, 1.19, 1.81, 1.85, 1.98, and 2.01 min, correspondingly. Mass spectrometry (MS) was connected to liquid chromatography with TSQ Quantis triple quadrupole mass spectrometer (Thermo Scientific, USA). The mass spectrometry condition was set to positive ion electrospray ionization (+ESI). The spraying voltage was stable at 3500 V under N2 Sheath. The controlled sheath, auxiliary, and sweep gases were 50, 10, and 1 Arbs. The precise temperatures of 325°C and 350°C condition were marked for ion transfer tube (ITT) and vaporizing conditions. The selected reaction monitoring (SRM) condition was the specific mode for mass analysis in the concurrent investigation of all masses. The mass-to-charge ratio (*m/z*) of valine precursor and quantified product masses were 118 and 72, separately. The transition was carried at an exact collision energy of 10 V. Masses were confirmed with defragmented mass product mass for valine was *m/z* 55 with a collision energy of 21 V for the transition. The mass-to-charge ratio (*m/z*) of ^{13}C/ ^{15}N-valine precursor and exact quantified defragmented mass was 306.3 and 262.3, correspondingly. The collision energy applied for the transition was 11 V for valine. The mass-to-charge ratio (*m/z*) of isoleucine precursor and quantified product masses was 132 and 86, correspondingly. A collision energy of 10 V was used for the transition. The confirmation product mass for isoleucine was m/z 69 with a collision energy of 17 V for the transition. The mass-to-charge ratio (*m/z*) of ^{13}C/ ^{15}N-isoleucine precursor and quantified product form was 139.2 and 74, individually. The collision energy of 18 V was used for the transition. The mass-to-charge ratio (*m/z*) of leucine precursor and quantified product atomic mass was 132 and 86, separately. The collision energy applied for the transition was 10V for leucine. The confirmation product mass for isoleucine was *m/z* 44 with the collision energy of the transition. The collision energy applied for the transition was 22 V for isoleucine. The mass-to-charge ratio (*m/z*) of ^{13}C/ ^{15}N-leucine precursor and quantified product form was 139.2 and 92, correspondingly. The collision energy of applied for the transition was 18 V for valine. The mass-to-charge ratio (*m/z*) of 13C/15N-valine precursor and quantified product form was 139.2 and 92, distinctly.

Analysis of plasma carnosine, β-alanine, and histidine via LC-MS/MS

Plasma carnosine, β-alanine, and histidine levels were determined by using a method described previously (31). Before using plasma samples, they were fully thawed by leaving them at room temperature. Sample preparation begins by combining 400 μL of methanol with 100 μL of plasma. Then, the mixed solution was centrifuged at 13,000 rpm for 15 min. The concentrated sample was evaporated with a SpeedVac concentrator to remove the solvent. The mobile phase A was prepared from 0.1% formic acid, which was applied to adjust the volume of the sample to a final capacity of 50 μL as reconstitution. The final solution was filtered through a 0.2 μm Nylon filter. Samples were kept in a glass ampoule for chemical evaluation. The liquid chromatography was processed using Ultimate 3000 Ultra High-performance liquid chromatography (UHPLC, Thermo Scientific, Waltham, MA, USA) well-assembled with a Hypersil GOLD^{TM} C18 column (100 × 2.1 mm, particle size 1.9 μm) at 30°C. Mobile phase solution for chromatography was prepared with MS-grade water with 0.1% formic acid (mobile phase A) and acetonitrile with 0.1% formic acid (mobile phase B). The analytical solution was controlled at a flow rate of 0.2 mL/min and a run time of 5 min per injection. The individual volume was 5 μL per single injection. The retention time was noticed. The adjusted gradient of mobile phases A and B by time laps is presented in Supplementary Table S2.

Mass spectrometry was connected to liquid chromatography with TSQ Quantis triple quadrupole mass spectrometer (Thermo Scientific, USA). The MS condition was set to positive ion electrospray ionization (+ESI) to identify mass spectra and transitions of carnosine, β-alanine, and histidine. The spraying voltage was stable at 4600V under N2 Sheath. The controlled sheath and auxiliary gases were 50 and 10 Arbs. The precise temperatures of 300°C and 350°C condition were marked for ion transfer tube (ITT) and vaporizing conditions. For the concurrent investigation of all masses, the selected reaction monitoring (SRM) condition was the specific mode for mass analysis. The mass-to-charge ratio (*m/z*) of β-alanine precursor and quantified product masses was 90.31 and 29.964, separately. The transition was carried at an exact collision energy of 13.76V. The mass-to-charge ratio (*m/z*) of histidine precursor and quantified product masses was 156.062 and 82.833, separately. The transition was carried at an exact collision energy of 24.29V. The confirmed fragment mass of 110 was further analyzed by applying a collision energy of 13.72. The mass-to-charge ratio (*m/z*) of carnosine precursor and quantified product masses was 227.2 and 110.054, separately. The transition was carried at an exact collision energy of 22.355 V. The confirmed fragment mass of 156.125 was further analyzed by applying a collision energy of 15.236.

Analysis of IL-6 and IL-10

Serum preparation for interleukin evaluation via ELISA

The blood samples were obtained in a serum vacutainer tube containing a clot activator, after overnight fasting. After the blood was clotted, each sample was centrifuged at controlled room air condition of 1,000 rpm for 10 min. The lower layer of solution was collected after centrifugation. Serum was processed and stored at −20°C until evaluation. The prepared serum was thawed by leaving it at room temperature before being used.

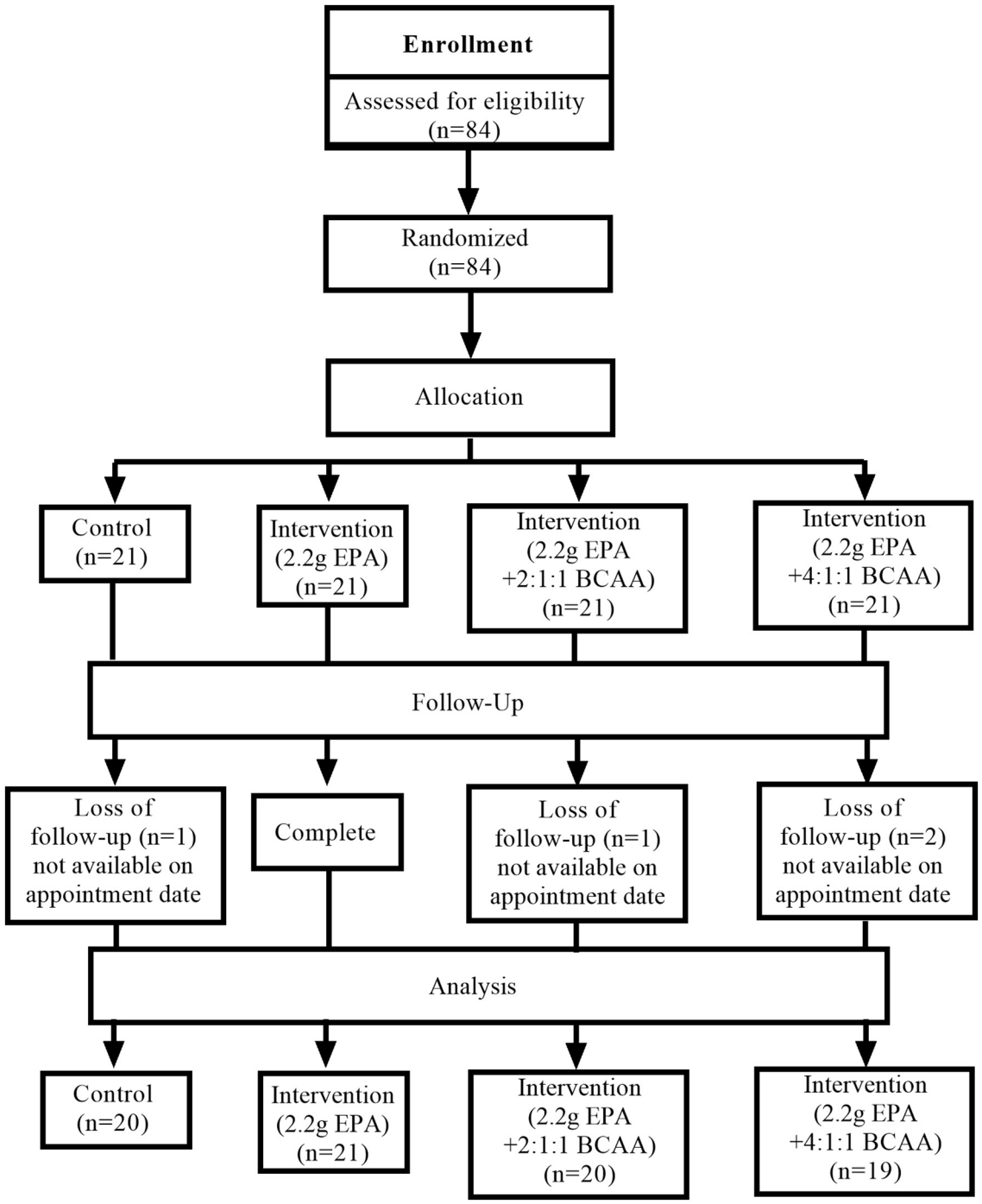

FIGURE 1
Conceptual framework.

ELISA procedure for the analysis of IL-6 and IL-10

The concentrations of human IL-6 and IL-10 within serum sample were evaluated by using the method of enzyme-linked immunosorbent assay (ELISA) kits (Catalog No. ab46042 and ab46034, correspondingly; Abcam Co., Cambridge, MA, USA), as described previously (32). The assays were performed according to the kits' instructions. For the test, 100 μL each of the given standard or prepared plasma samples was added to each well of the 96-well plate coated with the antibody of IL-6 or anti–IL-10. Then, 50 μL of biotinylated anti–IL-6 or anti–IL-10 antibody, which accounted for the secondary antibody, was added to the well, and the mixture was incubated for 2 h at room temperature. After that,

TABLE 1 Baseline characteristics of participants.

Characteristic	Number (%)				*p*-value
	Group 1 control ($n = 20$)	Group 2 EPA ($n = 21$)	Group 3 EPA+BCAA (2:1:1) ($n = 20$)	Group 4 EPA+BCAA (4:1:1) ($n = 19$)	
Age	67.86 ± 4.62	65.15 ± 4.09	68.05 ± 5.11	66.60 ± 5.28	0.24
Sex Male Female	4 (19.05%) 17 (80.95%)	4 (20%) 16 (80%)	4 (21.06%) 15 (78.94%)	4 (20%) 16 (80%)	0.93
Systemic diseases None DM/ DM+DLP DLP	11 (52) 4 (19) 6 (29)	11 (55) 3 (15) 6 (30)	8 (42) 3 (16) 8 (42)	8 (40) 5 (25) 7 (35)	0.13
Medication use None Yes[a]	11 (52) 10 (42)	11 (55) 9 (45)	8 (42) 11 (58)	8 (40) 12 (60)	0.88
BMI	22.9 ± 4.1	23.1 ± 4.6	22.2 ± 4.8	22.1 ± 3.9	0.93
Muscle mass (kg)	37.8 ± 8.1	37.3 ± 5.9	35.9 ± 5.7	37.8 ± 6.9	0.78
% Fat	28.6 ± 7.4	30.3 ± 9.8	27.8 ± 9.1	26.1 ± 9.6	0.50
Handgrip strength (Right arm)	22.2 ± 5.8	24.0 ± 5.5	22.8 ± 5.9	25.0 ± 5.3	0.28
Handgrip strength (Left arm)	21.2 ± 6.5	21.9 ± 6.6	21.7 ± 4.3	24.1 ± 5.7	0.40
Average energy intake	1226 ± 185.6	1300 ± 318.9	1192 ± 193.5	1201 ± 469.5	0.68
Average protein intake	52.97 ± 12.13	55.41 ± 12.40	52.09 ± 14.09	57.89 ± 19.60	0.61

Data were expressed as mean ± standard deviation (SD). The statistical differences among groups were analyzed using one-way ANOVA. [a]Blood sugar control/ blood lipid control drugs.

the samples were washed three times with 300 μL of wash buffer. Next step, 100 μL of Streptavidin-HRP solution was added into the previous mixture. The solution was incubated for 30 min. After three washes with buffer, 100 μL of chromogen TMB substrate was added, and the samples were incubated in the dark for 15 min. Finally, 100 μL of stop reagent was added to end the fraction. The concentrations were determined by a microplate reader capable of measuring absorbance by setting the wavelength at 450 nm (Epoch, BioTek Industries, Highland Park, USA). Calibration curves of IL-6 ranging from 0 to 50 mg/L were used for the quantitation of the IL-6 level in serum. Calibration curves of IL-10 ranging from 0 to 400 mg/L were used for the quantitation of IL-10 level in serum.

Statistical analysis

A per-protocol analysis was performed. Graphing and the following statistical analyses were conducted using Graph Pad Prism Software V. 7. Demographic characters between study and control groups were analyzed using the chi-square test. All parameters were compared between baseline and after intervention in the same group by using paired *t*-test or Wilcoxon signed rank test depending on the normality of the data. Comparison of changes over time of each parameter between study and control groups was performed using two-way repeated measure ANOVA followed by Tukey's multiple comparison tests.

Results

Participants' flow diagram

This study was conducted from August 2020 to July 2021. Figure 1 shows the Consolidated Standards of Reporting Trials (CONSORT) participants' flow diagram. Eighty-four volunteers were recruited for the study and all of them passed the screening. All 84 participants were randomly assigned into four groups (n = 21, for each group) receiving different medical formulas; (1) control complete nutrition drink, (2) fortified with only 2.2g/day of EPA, (3) fortified with 2.2g/day of EPA and 5g/day of BCAA 2:1:1 of Leu: Ile: Val, and (4) fortified with 2.2g/day of EPA and 5g/day of BCAA 4:1:1 of Leu: Ile: Val. Few participants in certain groups lost follow-up. The final data were collected from 20, 21, 20, and 19 participants in groups 1, 2, 3, and 4, respectively.

Participants' characteristics

The baseline characteristics of participants in each group are shown in Table 1. The mean ages of participants were 67.86 ± 4.62, 65.15 ± 4.09, 68.05 ± 5.11, and 66.60 ± 5.28 years old for groups (1), (2), (3), and (4), respectively. There was no significant difference in age among groups ($p = 0.239$). Most of the participants were in a greater proportion of females than males. There was no significant difference in gender distribution among groups ($p = 0.931$). The mean initial body mass indices

TABLE 2 Comparison of body weight, BMI, muscle mass, fat mass, and visceral fat before (visit 1) and after (visit 2) receiving the assigned drinks and handgrip practice for 3 weeks.

Body Composition	Group 1 control ($n = 20$)			Group 2 EPA ($n = 21$)			Group 3 EPA+BCAA (2:1:1) ($n = 20$)			Group 4 EPA+BCAA (4:1:1) ($n = 19$)		
	Visit 1	Visit 2	*p*-value	Visit 1	Visit 2	*p*-value	Visit 1	Visit 2	*p*-value	Visit 1	Visit 2	*p*-value
Body weight	56.6 ± 13	57.1 ± 13.1	0.10	57.5 ± 11.4	58.2 ± 11.4	**	56.4 ± 14.5	57.1 ± 14.3	**	55.6 ± 11.9	56.1 ± 12.0	0.11
BMI (kg/m^2)	23.0 ± 4.2	23.1 ± 4.3	0.58	23.1 ± 4.6	23.5 ± 4.5	*	22.4 ± 4.8	22.7 ± 4.7	0.19	22.2 ± 3.7	22.3 ± 3.8	0.93
Muscle mass (kg)	37.7 ± 8.3	36.4 ± 9.1	0.64	37.3 ± 5.9	37.6 ± 5.7	0.99	36.1 ± 5.8	38.3 ± 7.7	0.21	38.0 ± 7.2	39.1 ± 7.3	0.82
Right arm muscle mass (kg)	1.85 ± 0.55	1.9 ± 0.57	0.91	1.84 ± 0.43	1.9 ± 0.47	0.63	1.86 ± 0.52	1.93 ± 0.55	*	1.85 ± 0.53	1.93 ± 0.54	**
Left arm muscle mass (kg)	1.76 ± 0.53	1.77 ± 0.54	0.99	1.71 ± 0.41	1.77 ± 0.42	0.14	1.73 ± 0.51	1.8 ± 0.51	0.08	1.73 ± 0.49	1.79 ± 0.51	0.2
% Fat	28.62 ± 7.37	27.76 ± 9.76	0.64	30.3 ± 9.8	30.63 ± 9.5	0.98	27.83 ± 9.28	27.66± 9.31	0.99	25.98 ± 9.39	26.28 ± 9.09	0.99
Visceral fat rating	8.25 ± 4.78	8.1 ± 4.72	0.94	7.9 ± 3.3	8.04 ± 3.35	0.95	7.7 ± 4.54	7.9 ± 4.42	0.86	7.95 ± 5.01	8.42 ± 4.98	0.19

Data were expressed as mean ± standard deviation (SD). The statistical differences between visit 1 (baseline) and visit 2 (3 weeks after intervention) for each group were analyzed using two-way repeated measure ANOVA followed by Sidak's multiple comparison tests (comparing different time points in the same group). The symbols * and ** mean $p < 0.05$ and 0.01, respectively.

were not different among groups ($p = 0.929$). Furthermore, there were no significant differences among groups for systemic diseases, medications, muscle mass, % fat, handgrip strength, and average energy and protein intakes.

Changes in body weight, BMI, muscle mass, fat mass, and visceral fat

After participants drank the given complete nutrition drinks of each group along with performing the hand grip exercise, all of them were measured for the changes in body composition via BIA. The changes in body weight, BMI, muscle mass, fat mass, and visceral fat before and after receiving complete nutrition drinks from each group of participants are shown in Table 2. The body weight of groups 2 and 3 and the BMI of group 2 increased significantly after consuming the assigned interventions ($p < 0.01$ and $p < 0.05$, respectively). Interestingly, as shown in Table 2, the average right arm muscle mass was slightly increased in all groups. The statistically significant improvement was only observed in groups 3 and 4, which received complete nutrition drink fortified with 2.2 g/day of EPA and 5 g/day of BCAA 2:1:1 and 4:1:1 of Leu: Ile: Val, respectively ($p < 0.05$ and $p < 0.01$, respectively). No significant differences in total muscle mass, left arm muscle mass, % fat, and visceral fat rating were found between before and after interventions. When comparing changes (as % baseline) after interventions among groups, there were no statistically significant differences in body weight, BMI, resting energy expenditure (REE), total body water, visceral fat rating, total, left and right arm muscle mass, total, and left and right arm fat mass among all groups (Figures 2–4). Interestingly, Figure 3 shows a pronounced higher increase in the total muscle mass and the right arm muscle mass after receiving the fortified formula with EPA and 5g/day of BCAA 4:1:1 of Leu: Ile: Val. However, two-way ANOVA showed no statistically significant difference from other groups.

Changes in handgrip strength

During the trial, muscle training with a resistance rubber handgrip was assigned for all participants to perform daily. Moderate training on hands and arm muscles was assigned since it has a low risk of falls in the elderly. To avoid inconsistency, all individuals were asked to continue their routine exercise without extreme exercise throughout the study. A comparison of handgrip strength changes within each group is shown in Table 3. Similar to the result of the right arm muscle mass, a significant increase in handgrip strength of the right arm was found in groups 3 and 4 after consuming the complete nutrition drink fortified with 2.2 g/day of EPA and 5 g/day of BCAA 2:1:1 and 4:1:1 of Leu: Ile: Val, respectively ($p < 0.05$ and $p < 0.01$, respectively). Interestingly, Figure 5 shows a higher increase in the right handgrip strength after receiving the fortified formula with EPA and 5g/day of BCAA 4:1:1 of Leu: Ile: Val. However, two-way ANOVA showed no statistically significant difference from other groups.

Changes in nutrition intake

All of the volunteers were asked to record the daily intake of regular food with assigned complete nutrition drinks. The calculated compliance to the given nutrition formula of groups 1–4 was 81.90, 84.05, 79.76, and 79.88%, respectively, which indicated a non-significantly difference (p=0.647). The nutritional values were calculated from the 3-day record per week to evaluate the differences in intakes before and after the trial. The energy intake and the intakes of macronutrients are significantly greater than

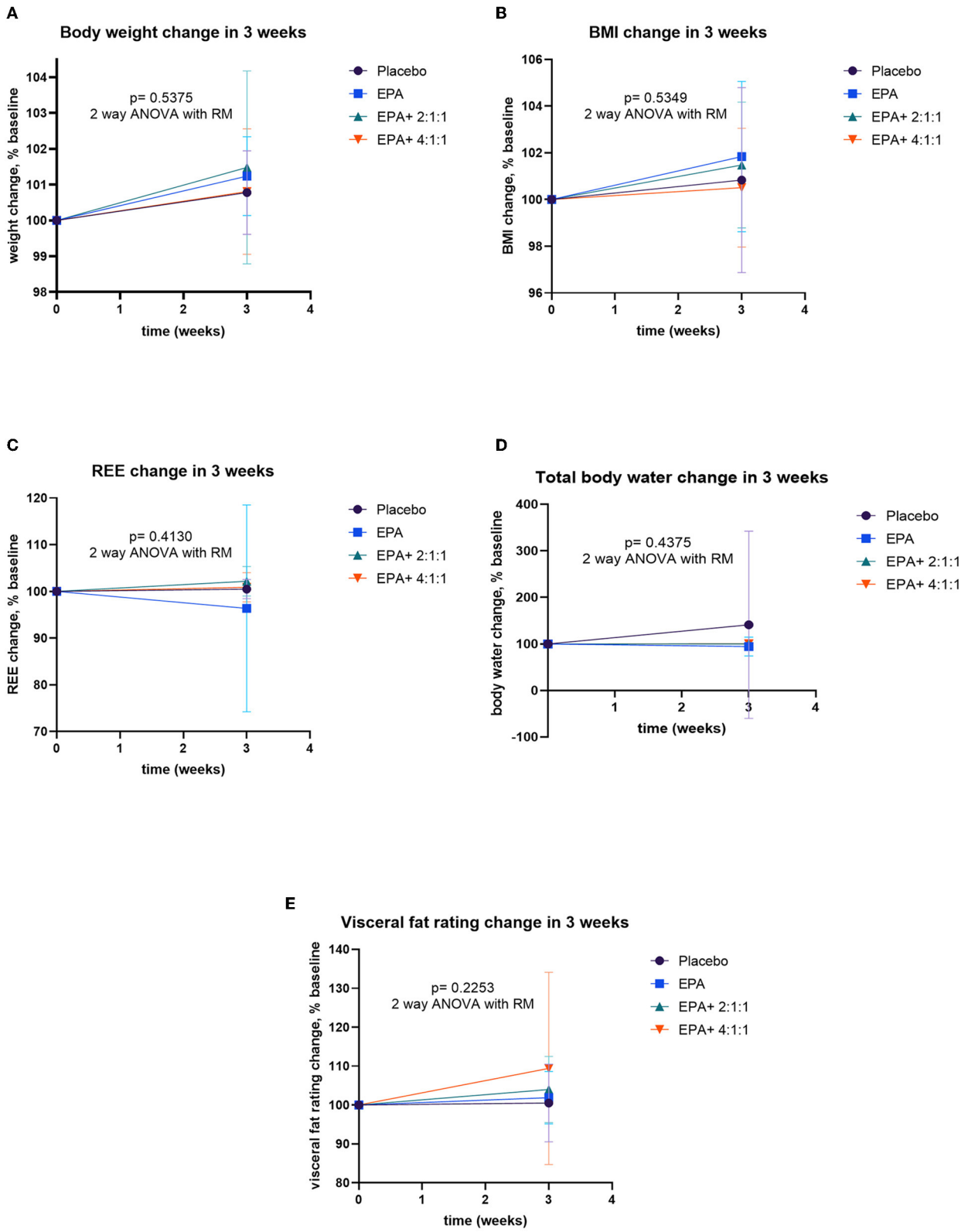

FIGURE 2
Percent changes of body composition before and after receiving specified interventions for 3 weeks. Line plots show mean and standard deviation (SD) of body weight **(A)**, BMI **(B)**, resting energy expenditure **(C)**, water **(D)**, and visceral fat **(E)**. The differences among groups were analyzed using two-way ANOVA with repeated measure.

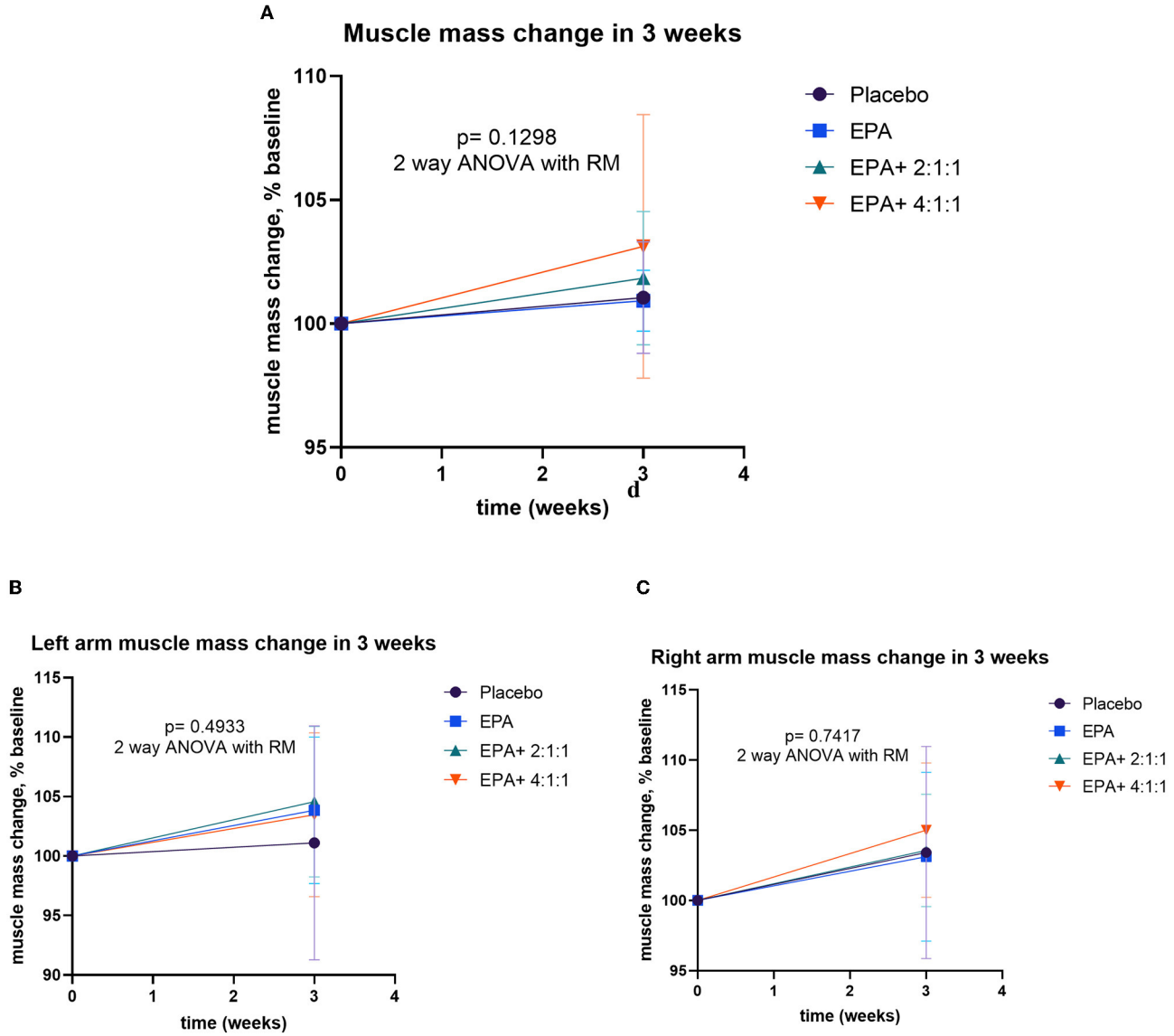

FIGURE 3
Percentage changes in muscle mass, left arm muscle mass, and right arm muscle mass before and after receiving specified interventions for 3 weeks. Line plots show the mean and standard deviation (SD) of total muscle mass **(A)**, left arm muscle mass **(B)**, and right arm muscle mass **(C)**. The differences among groups were analyzed using two-way repeated measure ANOVA.

those of baseline values in all groups (p=0.015, 0.012, 0.029, and 0.033 for groups 1–4, respectively). The average intakes are expressed in Supplementary Table S3. The comparison of dietary changes among groups was analyzed by using repeated measure ANOVA. The results showed non-significant differences among groups (Figure 6).

Changes in blood chemistry

The routine blood chemistry laboratory analysis was performed to monitor safety. As shown in Supplementary Table S4, blood biochemical values in all groups were not significantly altered.

Changes in plasma EPA and BCAA

The blood samples were drawn at the baseline and after continuous consumption of the assigned complete nutrition drinks for 3 weeks. Plasma was prepared for the analysis of EPA and BCAA by using LC-MS/MS. The comparison of plasma EPA and BCAA concentration via LC-MS/MS within each group is expressed in Table 4. For the non-hydrolyzed EPA evaluation, there was significantly elevated EPA in group 2 after receiving fortified formula containing EPA (p=0.0002) and in group 4 after receiving fortified formula containing EPA + 4:1:1 BCAA ($p < 0.0001$). Moreover, the significant elevation of hydrolyzed EPA was evidenced in group 2 ($p = 0.078$) and group 4 ($p < 0.0001$), respectively. The BCAA was significantly increased

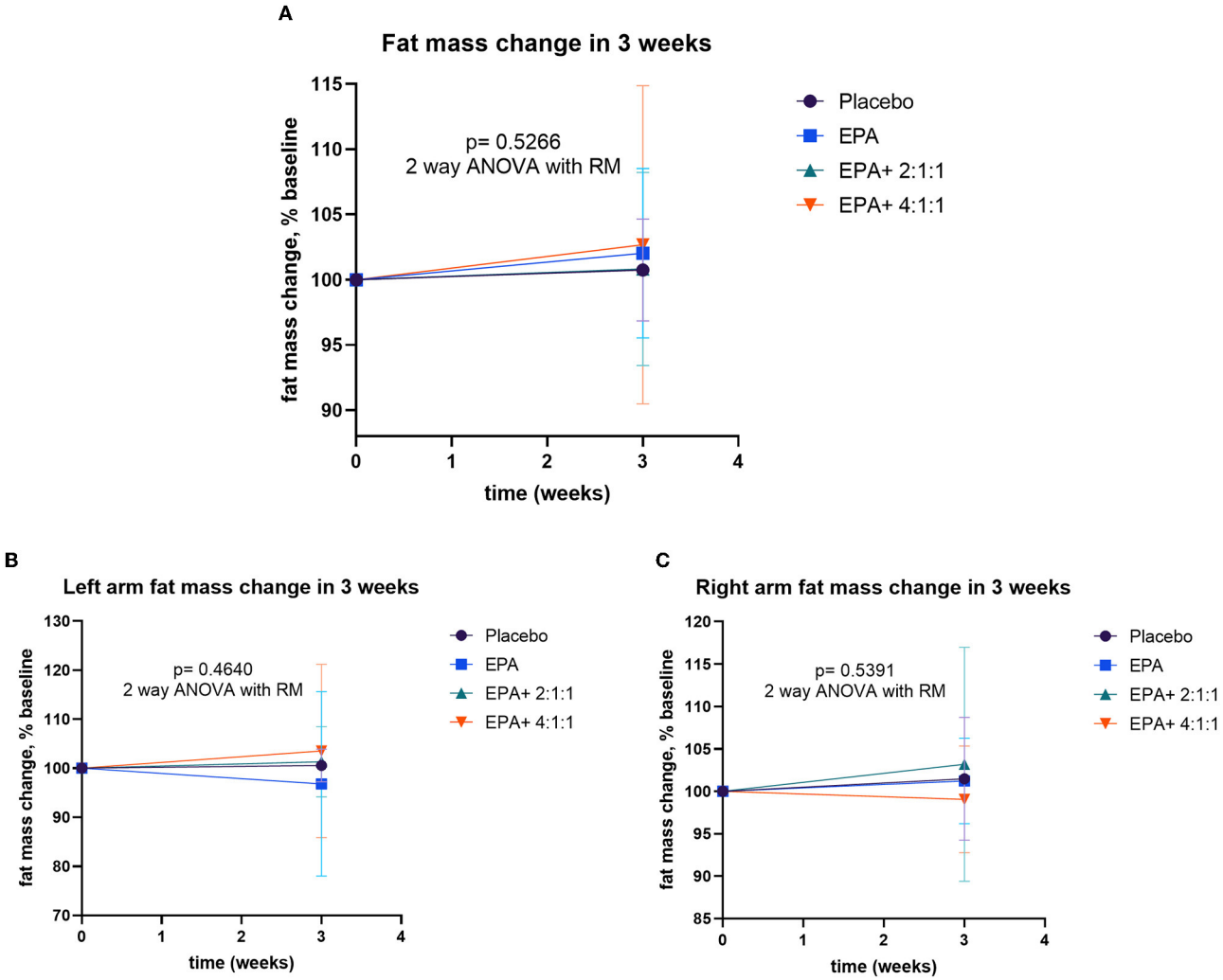

FIGURE 4
Percentage changes in fat mass, left arm fat mass, and right arm fat mass before and after receiving specified interventions for 3 weeks. Line plots show mean and standard deviation (SD) of total fat mass **(A)**, left arm muscle mass **(B)**, and right arm muscle mass **(C)**. The differences among groups were analyzed using two-way repeated measure ANOVA.

in all groups, compared to that of the baseline. Figure 7 shows the comparison of EPA and BCAA among groups. A significant elevation in non-hydrolyzed EPA was observed in the group consuming formula with EPA and 4:1:1 BCAA ($p = 0.036$). The same group also had significantly higher levels of plasma leucine and isoleucine than those of other groups ($p = 0.001$ and $p = 0.014$, respectively).

Changes in plasma carnosine, β-alanine, and histidine

Changes in muscle protein synthesis/ degradation were evaluated by analyzing the amount of plasma carnosine, β-alanine, and histidine. An increase in carnosine indicates a shift toward synthesis, while an increase in β-alanine or histidine represents more muscle protein breakdown. The analysis of plasma carnosine, β-alanine, and histidine concentration via LC-MS/MS within each group is shown in Table 5. Since all data of β-alanine were lower than the lowest quantitation (LOQ) level, the exact amount could not be calculated and reported here. Results of carnosine and histidine showed non-significant changes among groups (Figure 8). Interestingly, Figure 8A shows that group 4 receiving fortified formula with EPA+4:1:1 BCAA has a tendency of increased carnosine level, compared to other groups. Moreover, Figure 8B shows that the plasma histidine levels of the groups receiving fortified formula tended to decrease compared to the placebo control.

Changes in serum IL-6 and IL-10

The serum of the participants was separated from a blood sample and examined for inflammation markers via ELISA. The IL-6 represented an inflammation marker, while IL-10 signified

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TABLE 3 Comparison of handgrip strength within each group.

Handgrip strength*	Group 1 control			Group 2 EPA			Group 3 EPA+BCAA (2:1:1)			Group 4 EPA+BCAA (4:1:1)		
	Visit 1	Visit 2	*p*-value	Visit 1	Visit 2	*p*-value	Visit 1	Visit 2	*p*-value	Visit 1	Visit 2	*p*-value
Right arm	22.19 ± 5.84	23.33 ± 5.45	0.051	23.98 ± 5.5	24.88 ± 5.84	0.14	22.77 ± 5.97	23.92 ± 5.87	*	25.03 ± 5.32	26.57 ± 5.62	**
Left arm	21.18 ± 6.60	22.42 ± 6.30	0.12	21.86 ± 6.63	22.07 ± 5.91	0.99	21.78 ± 4.39	22.80 ± 5.02	0.27	24.44 ± 5.88	25.35 ± 5.23	0.40

Data were expressed as mean ± standard deviation (SD). The statistical differences between visit 1 (baseline) and visit 2 (3 weeks after intervention) for each group were analyzed using paired t-tests. Each parameter was the average parameter of three times repeated grip measurements. The symbols * and ** mean $p < 0.05$ and 0.01, respectively.

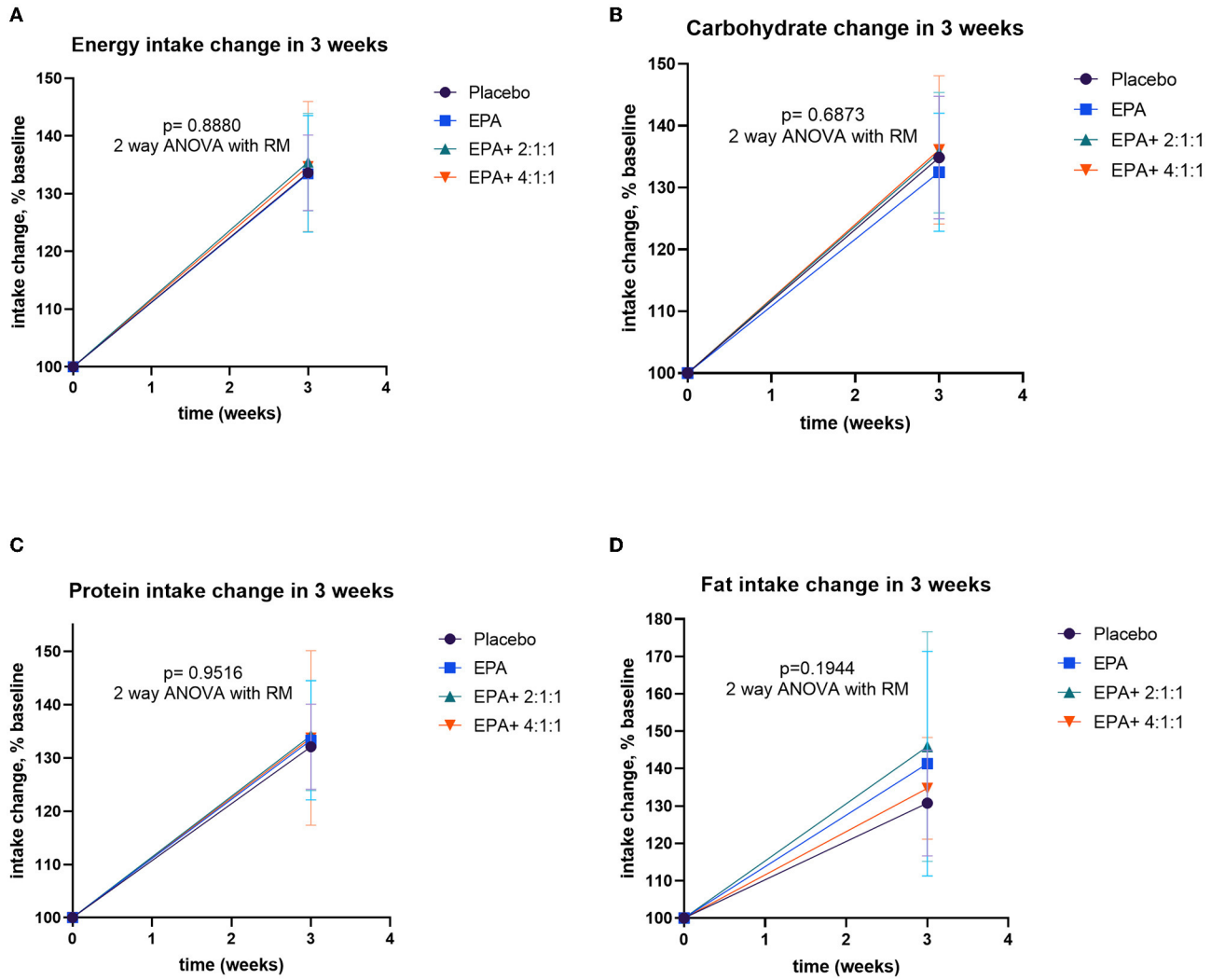

FIGURE 5
Changes in intakes including energy, carbohydrate, protein, and fat before and after receiving specified interventions for 3 weeks. Line plots show mean and standard deviation (SD) of changes (% of baseline values) in energy **(A)**, carbohydrate **(B)**, protein **(C)**, and fat **(D)** intakes. The statistical differences among groups were analyzed by using two-way repeated measure ANOVA.

an anti-inflammation marker. As shown in Figures 9A, C average IL-6 levels were decreased after receiving fortified formula with EPA+4:1:1 BCAA. However, the difference between the placebo and other groups was not statistically significant. As shown in Figures 9A, C average IL-6 levels were decreased after receiving fortified formula with EPA+4:1:1 BCAA. However,

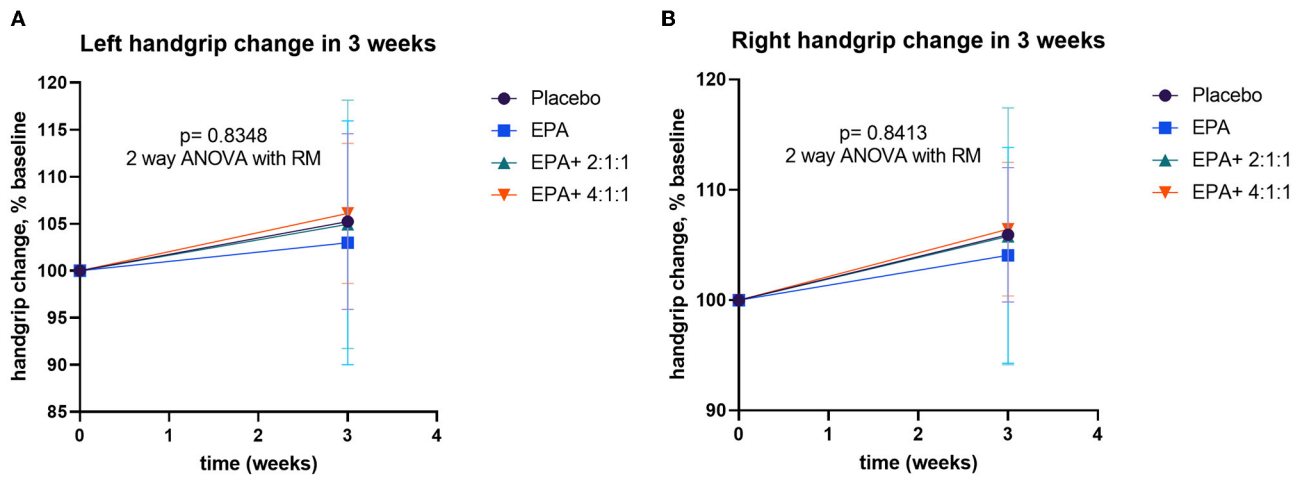

FIGURE 6
Percentage changes in handgrip strength before and after receiving specified interventions for 3 weeks. Line plots show the mean and standard deviation (SD) of change (% baseline) of the left **(A)** and right **(B)** handgrip strength. The differences within group were analyzed using two-way repeated measure ANOVA.

TABLE 4 Comparison of plasma EPA and BCAA concentrations via LC-MS/MS within each group.

Chemical analyze	Group 1 control			Group 2 EPA			Group 3 EPA+BCAA (2:1:1)			Group 4 EPA+BCAA (4:1:1)		
	Visit 1	Visit 2	*p*-value	Visit 1	Visit 2	*p*-value	Visit 1	Visit 2	*p*-value	Visit 1	Visit 2	*p*-value
Non-hydrolyzed EPA	16.90 ± 18.17	78.39 ± 112.0	0.530	15.73 ± 17.02	165.20 ± 184.7	0.0002	6.74 ± 5.63	101.60 ± 126.2	0.093	8.44 ± 6.99	172.70 ± 147.80	<0.0001
Hydrolyzed EPA	9.34 ± 9.60	688.70 ± 839.0	0.275	9.31 ± 7.86	871.60 ± 1173	0.078	8.33 ± 5.94	784.30 ± 729.0	0.182	5.34 ± 2.83	1551 ± 2100	<0.0001
Leu	165.60 ± 50.05	337.60 ± 124.6	<0.0001	154.20 ± 43.78	277.60 ± 72.45	0.002	142.70 ± 75.09	348.20 ± 132.9	<0.0001	129.10 ± 62.59	398.20 ± 151.8	<0.0001
Ile	33.62 ± 11.85	58.62 ± 18.29	0.0007	32.28 ± 8.65	62.77 ± 12.51	<0.0001	35.80 ± 13.77	75.73 ± 34.76	<0.0001	29.04 ± 14.23	69.53 ± 23.33	<0.0001
Val	117.60 ± 23.16	204.30 ± 83.41	0.008	127.30 ± 24.23	225.60 ± 90.82	0.002	111.70 ± 41.95	232.80 ± 112.4	<0.0001	120.90 ± 27.62	250.80 ± 125.0	<0.0001

Data were expressed as mean ± standard deviation (SD). The statistical differences between visit 1 (baseline) and visit 2 (3 weeks after intervention) for each group were analyzed using paired t-tests.

the change was not statistically significantly different from the placebo and other groups. Furthermore, as shown in Figures 9B, D all study groups receiving the drinks fortified with EPA with or without BCAA have a non-significant trend of increased IL-10 plasma levels compared to those of placebo control.

Discussion

Elevated inflammation and negative nutritional balance are demonstrated to be major causes of developing sarcopenia (13). Nevertheless, it is unknown if energy supplementation plus the combination of anti-inflammatory factors such as eicosapentaenoic acid (EPA) and branched-chain amino acids (BCAAs) would be effective for the prevention of sarcopenia in the elderly with inadequate protein intake. This 3-week randomized control trial revealed that consuming a complete nutrition drink fortified with EPA + BCAA 2:1:1 and 4:1:1 of Leu: Ile: Val for 3 weeks significantly increased the right arm muscle mass and right handgrip strength ($p < 0.05$ and $p < 0.01$, respectively) along with non-significantly elevated carnosine with reduced histidine and increased IL-10 with decreased IL-6 levels. Previous studies showed a beneficial effect of EPA on anti-inflammation and the effect of BCAA supplementation on muscle health. Here we showed that a combination of both could have a trend in improving muscle health and reducing inflammation. This short-term study implies the potential benefits of such a combinatorial approach in preventing sarcopenia in the elderly with inadequate protein intake.

Our finding is consistent with a previous systemic review of 14 randomized controlled trials that show significantly improved

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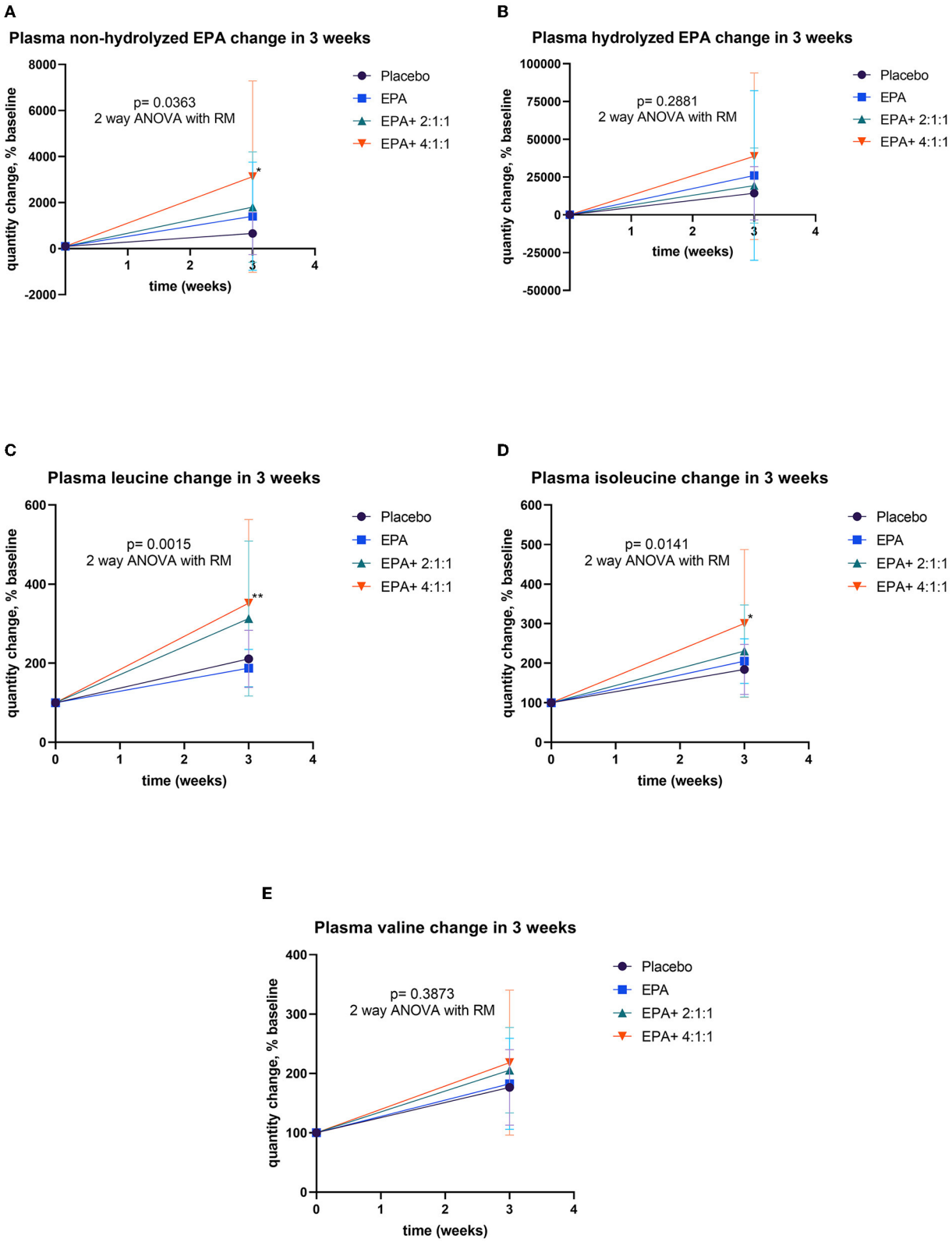

FIGURE 7
Percentage changes of plasma EPA and BCAA before and after receiving specified interventions for 3 weeks. Line plots show the mean and standard deviation (SD) of change (% baseline) of plasma non-hydrolyzed EPA **(A)**, hydrolyzed EPA **(B)**, leucine **(C)**, isoleucine **(D)**, and valine **(E)**. The statistical differences among groups were analyzed using two-way ANOVA with repeated measure.

TABLE 5 Comparison of plasma carnosine, β-alanine, and histidine concentrations via LC-MS/MS within each group.

Chemical analyze	Group 1 control			Group 2 EPA			Group 3 EPA+BCAA (2:1:1)			Group 4 EPA+BCAA (4:1:1)		
	Visit 1	Visit 2	*p*-value	Visit 1	Visit 2	*p*-value	Visit 1	Visit 2	*p*-value	Visit 1	Visit 2	*p*-value
Carnosine	17.72 ± 4.83	17.29 ± 2.51	0.997	16.50 ± 0.38	16.79 ± 0.73	0.999	16.62 ± 0.44	16.87 ± 0.66	>0.0.999	16.55 ± 0.29	17.23 ± 0.91	0.964
Histidine	199.00 ± 104.00	216.90 ± 117.20	0.999	198.40 ± 104.00	224.00 ± 112.70	0.996	215.90 ± 103.60	239.20 ± 113.40	0.998	210.80 ± 112.70	228.20 ± 124.50	0.999
β-alanine	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ

Data were expressed as mean ± standard deviation (SD). The statistical differences between visit 1 (baseline) and visit 2 (3 weeks after intervention) for each group were analyzed using paired t-tests.

muscle mass and muscle strength in elderly people after receiving BCAA-rich nutrition supplementation (33). However, while the systemic review showed a significant effect on total muscle mass, we only see the efficacy on right arm muscle mass and handgrip strength. Though the combination showed better effects than those of the placebo and the EPA only, there were no significant differences between groups. The distinct between our study and those reported in systemic reviews may be due to two reasons; one is that our study population is just at risk of sarcopenia but had not been diagnosed as sarcopenia yet, and the other is the short duration of our study (3 weeks). Previous studies that show statistically significant effects on muscle mass, strength, and performance provided BCAA supplements for at least 5 weeks (34). In the current study of 3 weeks of intervention, a significant increase in muscle mass and handgrip strength of the right arm was observed after receiving a complete nutrition drink fortified with EPA + BCAA 2:1:1 and 4:1:1). This favorable effect may be explained by the combinatorial mechanisms of promoting muscle protein synthesis and prevention of muscle loss from inflammation. In this group, plasma EPA and BCAA levels were also increased significantly along with the tendency in increasing muscle protein synthesis marker - carnosine and anti-inflammatory marker - IL-10 and decrease muscle degradation marker - histidine and pro-inflammatory marker - IL-6.

Sarcopenia occurs through age-related deterioration in muscle protein synthesis along with chronic inflammation accelerating the process of muscle protein degradation (34). While pro-inflammatory cytokines such as IL-6, IL-1, and TNF-α induce inflammation and promote muscle degradation, anti-inflammatory cytokines such as IL-10 inhibit inflammation and prevent sarcopenia (35). Numerous studies have shown the association between elevated pro-inflammatory markers and reduced muscle mass and strength in the sarcopenic elderly (36). Using a combination between EPA and BCAA, we found a non-significant trend in the decrease of pro-inflammatory cytokines and an increase in anti-inflammatory cytokines, which is likely derived from the effect of EPA. A reduced inflammatory state may result in decrease muscle degradation as we observed a tendency in the decrease of free amino acid histidine after interventions. Furthermore, the tendency to increase muscle protein synthesis marker carnosine likely stems from the effect of BCAA. Taken together, the findings from this short-term study suggest that the combination of EPA and BCAA might have the potential to reduce inflammatory-related muscle protein degradation and increase muscle protein synthesis. Future long-term studies are warranted to investigate the anti-inflammatory and muscle synthesis-promoting effect of this EPA-BCAA combination. More inflammatory biomarkers including PAF and TNF-α should also be measured besides the interleukins. It is worth noting that the significant effect was observed only in the right arm but not the left arm or total body muscle mass. The reason for this finding may be explained by the arm exercise instructed in this study. Though we asked all participants to do the exercise in both arms, most participants are right-handed. Future studies are warranted to investigate further if full-body exercise could help improve total muscle mass.

A high BCAA and/or leucine content is generally required for stimulating protein synthesis in skeletal muscle tissue (37). Generally, amino acids serve as a substrate, but branch-chain amino acids especially leucine can directly activate muscle protein synthesis by activating the mechanistic target of rapamycin (mTOR) signaling (38, 39). Leucine was essential for triggering the mammalian target of rapamycin complex 1 (mTORC1), as well as the downstream phosphorylation of p70S6 kinase (p70S6k) and 4E (eIF4E)-binding protein 1 (4E-BP1) and related signaling pathways in muscle rejuvenation (38, 39). Interestingly, in the current study, we also found more favorable effects in the group receiving EPA + BCAA (4:1:1 of Leu: Ile: Val) than that of EPA + BCAA (2:1:1 of Leu: Ile: Val), suggesting the importance of leucine. However, the previous study highlighted that BCAA intake had to be consistent to successfully maintain muscle mass (33). According to repeated measures for plasma BCAA changes, we also observed significantly elevated plasma leucine and isoleucine in the group with EPA + BCAA (4:1:1). These outcomes confirm that the intervention really cause an increase in the bioavailable amino acids. Consistently, our finding suggests that supplementation with EPA + BCAA 2:1:1 and 4:1:1 for 3 weeks may improve right arm muscle mass and strength. However, the changes are still not significantly different from the placebo or EPA only. It is worth noting that in this study, we also observed the elevation of plasma BCAA after consuming the unfortified complete nutrition drink (placebo) and the one fortified with EPA only. The results may be because the complete nutrition drink can provide both energy and protein, which could be digested further to BCAA. The elevation of plasma BCAA in the placebo or

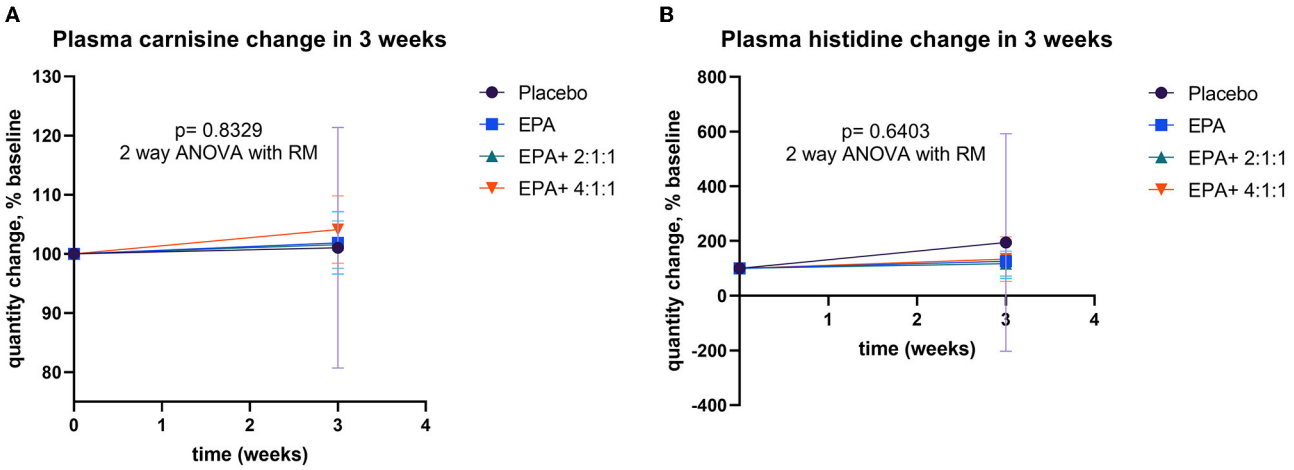

FIGURE 8
Percentage changes in plasma EPA and BCAA before and after receiving specified interventions for 3 weeks. Line plots show the mean and standard deviation (SD) of change (% baseline) in plasma carnosine **(A)**, and histidine **(B)**. The statistical differences among groups were analyzed using two-way repeated measure ANOVA.

EPA only may explain the non-statistically significant of right arm muscle mass and strength among different groups. Future studies with longer duration of intervention are warranted to investigate the effect of the EPA + BCAA combination.

A previous meta-analysis of 10 randomized control trials showed that omega-3 fatty acid supplements at more than 2 g/day may contribute to muscle mass gain (0.67 kg; 95% CI: 0.16, 1.18) and improve walking speed (1.78 m/sec; 95% CI: 1.38, 2.17), especially for those receiving more than 6 months of intervention (40). For example, Smith et al. showed that daily consumption of fish oil for 6 months resulted in improved muscle mass, handgrip strength, and muscle performance (41). In the current study of 3 weeks, combining 2 g/ day of EPA with 5 g/ day of BCAA in a complete nutrition drink plus hand exercise showed a significant increase in right arm muscle mass and strength along with non-significant elevation in muscle protein synthesis marker. The findings suggest that the combination may help facilitate the efficacy of a short-term intervention. A more recent meta-analysis found that omega-3 fatty acid supplements did not affect muscle mass but improved muscle strength and muscle performance in older adults (42). The potential mechanisms of omega-3 fatty acids to promote muscle mass and physical performance include anti-inflammatory effects, mTOR pathway, and reduction of insulin resistance (43). Its anti-inflammatory effect is most well-studied. In fact, a meta-analysis study confirmed that there was a reduction in inflammation markers (i.e., CRP and IL-6) after taking supplementation with omega-3 PUFAs in middle-aged and older adults (44).

Our current study shows the tendency of decreased IL-6 and increased IL-10 plasma levels after consuming fortified formula with EPA and EPA+BCAA (4:1:1). While the mechanisms underlying sarcopenia remained to be elucidated, chronic low-grade inflammation is one of the most documented mechanisms of sarcopenia. The elevations in cytokines (i.e., IL-6 and TNF-α) were found to correlate with functional disability and may be involved in sarcopenia through effects on pathways controlling protein metabolism (43). In this study, we found a significant elevated plasma EPA after consuming the complete nutrition drink with 2.2g EPA only, and the 2.2g EPA with BCAA (4:1:1). For comparison among groups, a significantly elevated plasma EPA was found in the non-hydrolyzed form in the group consuming 2.2g EPA with BCAA (4:1:1) compared to the placebo control (p=0.0003). The findings suggest that the addition of EPA in complete nutrition drinks could be a more promising available source of EPA than a regular diet with iso-caloric control drinks. In recent years, the n-3 PUFAs found in fish oil were studied to treat sarcopenia as an anti-inflammation related to the maintenance of muscle health in older adults (45). Primarily, a former study in community-dwelling elderly found that n-3 fatty acid intake was 19% different among elderly that were diagnosed with sarcopenia and non-sarcopenia ($p = 0.005$) (46). In 2017, a remarkable randomized control trial investigated the effects of EPA and DHA therapy on inflammation in older adults. Supplementation had a significant decreasing effect on IL-6, IL-1β, and TNFα levels after 4 weeks of use and was even greater after 8 weeks (47). An *in vitro* study in a mouse model highlighted novel pathways associated with lipotoxicity and cytotoxicity in the potential targeting of molecular modulators of sarcopenic obesity, with mice fed with EPA-rich food under cytotoxic stress (TNF-α) shown to partially rescue differentiation with enhanced myotube formation of mouse skeletal tissue (48). In our current study, although the inflammation markers did not express significant changes, a tendency of decreased pro-inflammatory cytokine such as IL-6 and an increased anti-inflammatory cytokine such as IL-10 was found, suggesting a potential reduction in inflammation. Future studies with longer duration of treatment may be required to see the significant anti-inflammatory effect of EPA in the elderly at the risk of sarcopenia.

Monitoring of muscle turnover with carnosine, histidine, and β-alanine was applied to measure the rate of protein

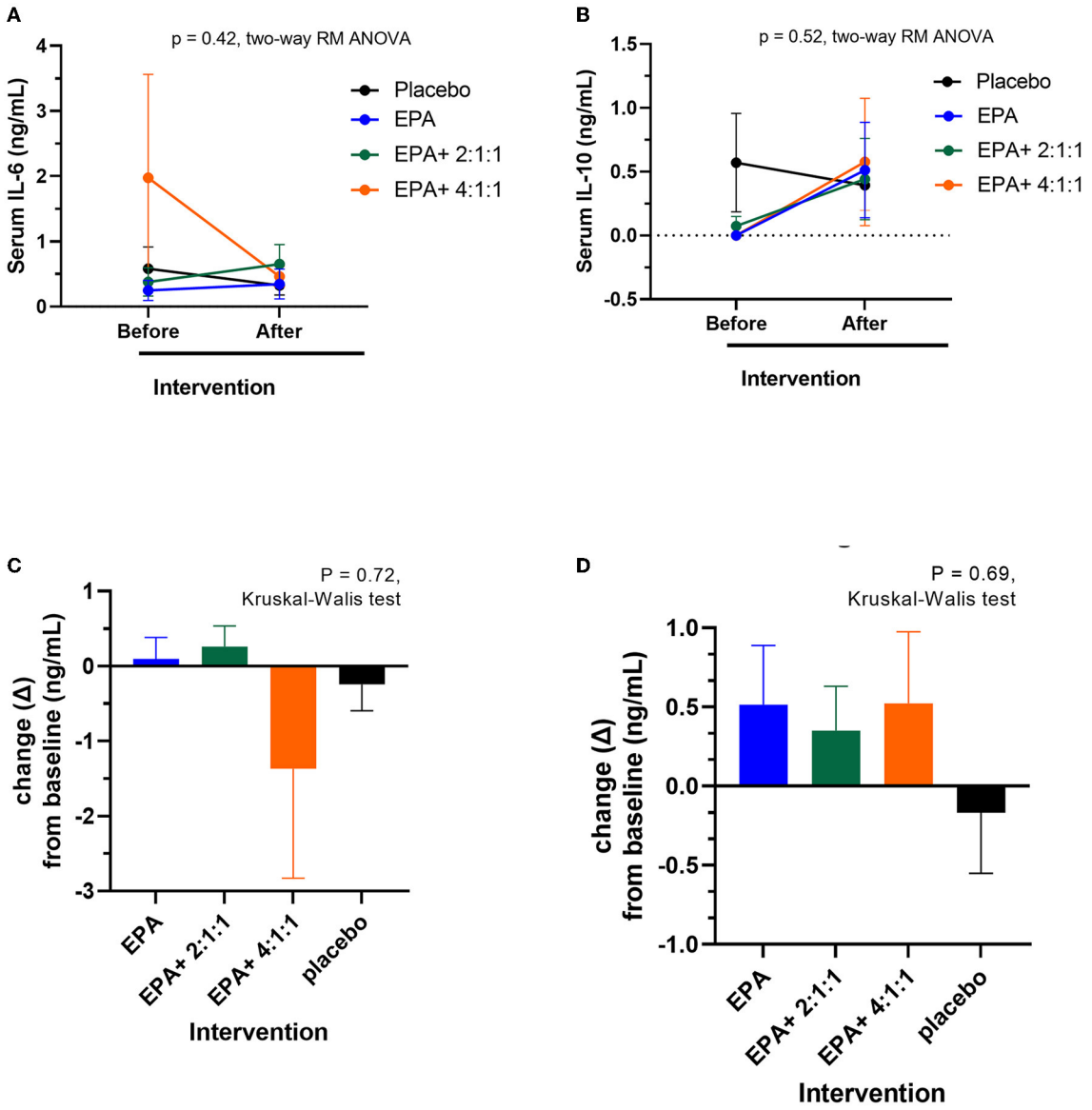

FIGURE 9
Changes in serum IL-6 and IL-10 levels before and after receiving specified interventions for 3 weeks. Line plots show the mean and standard deviation (SD) of serum IL-6 **(A)** and IL-10 **(B)**, changes (delta = the levels after intervention − the levels at baseline) of IL-6 **(C)** and IL-10 **(D)**. The statistical differences among groups were analyzed using two-way repeated measure ANOVA.

breakdown. A tendency of increased carnosine and decreased histidine levels was found after consuming fortified formula with EPA and BCAA (4:1:1), compared to other groups. An increase in dipeptide (carnosine) and a decrease in free amino acid (histidine) suggest a shift toward the anabolism of muscle protein. This finding is consistent with the observed effect on promoting muscle strength and muscle mass. Carnosine (β-alanyl-L-histidine) is an intramuscular dipeptide consisting of β-alanine and L-histidine (49). Therefore, the changes in the ratio between the free amino acids (β-alanine and histidine) and the dipeptide carnosine can be an indicator of changes in muscle protein synthesis and degradation. Moreover, a plasma lipid profile of lower n6, which antagonize n3, has been associated with reduced PAF biosynthesis and/or increased catabolism (21). However, this study did not find significant differences among groups of treatment. Future studies with a longer duration of consuming a fortified formula with EPA and BCAA (4:1:1) are warranted.

The strength of this study is the randomized placebo design with the comparison between EPA alone, EPA + BCAA at ratios 2:1:1 and 4:1:1 of Leu: Ile: Val. Furthermore, a significant increase in EPA, leucine, and isoleucine in the bloodstream assures effective supplementation and compliance. Compared to other studies, this study utilized a much shorter duration of treatment with BCAA but still can show some tendency of positive changes in all important parameters. Nevertheless, short-term supplementation (3 weeks)

and small sample size are limitations for this study to see a statistical difference between groups. Therefore, a longer period of the clinical trial in a larger sample size may be needed to investigate the effect of the EPA-BCAA combination. It was worth noting that the results of this study were summarized by the average values from all participants comparing the consequences of EPA and BCAA among groups of intervention. The actual results for each individual were varied. Future studies should be performed to characterize the responders and non-responders according to their background such as genetic polymorphisms. In future, we hope that the specific fortified complete nutrition drinks may be used for the precise person as the concept of personalized nutrition.

Conclusion

This 3-weeks clinical trial demonstrated that the EPA or EPA–BCAA combination fortified complete nutrition drinks were all well-tolerated. A significant improvement in right arm muscle mass and right handgrip strength was found after receiving a complete nutrition drink fortified with EPA and BCAA 2:1:1 and 4:1:1 of Leu: Ile: Val. However, the changes are not statistically different from those of control or EPA-only formulas. The plasma metabolite of EPA, leucine, and isoleucine was significantly increased among all interventions within 3 weeks. No significant changes in inflammatory cytokines and muscle degradation markers were observed. Nevertheless, the tendency of decreased inflammatory cytokines and increased inflammatory cytokines, decreased histidine, and increased carnosine was observed after consuming EPA and BCAA (4:1:1) compared to the placebo control group. This clinical trial suggested that the fortification of EPA together with a high proportion of leucine in BCAA (4:1:1) formula elevated available plasma EPA and BCAA. These findings suggest that consuming a complete nutrition drink fortified with EPA and BCAA 2:1:1 and 4:1:1 for 3 weeks might improve right arm muscle mass and strength likely by the tendency to promote muscle protein synthesis and anti-inflammatory effects. Future studies with longer duration are warranted to confirm it.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving human participants were reviewed and approved by the Mahidol University Central Institutional Review Board (MU-CIRB), Mahidol University, Thailand. The patients/participants provided their written informed consent to participate in this study.

Author contributions

WK: designed the manuscript, obtained ethical approval, collected data, performed laboratory analyses, statistical analyses, and drafted the manuscript. PS and CS: designed the manuscript and provided scientific input in the discussion of the data. KP: collected data. DT: obtained the grant, designed the manuscript, performed randomization, supervised laboratory analysis, and edited the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

DT received research grant from a governmental funding agency, Thailand Science Research and Innovation (TSRI), and a private company, Thai Otsuka Pharmaceutical Co., Ltd., Thailand.

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.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2023.1164469/full#supplementary-material

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Advances in nutritional supplementation for sarcopenia management

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Sarcopenia is a syndrome characterized by a decline in muscular mass, strength, and function with advancing age. The risk of falls, fragility, hospitalization, and death is considerably increased in the senior population due to sarcopenia. Although there is no conclusive evidence for drug treatment, resistance training has been unanimously recognized as a first-line treatment for managing sarcopenia, and numerous studies have also pointed to the combination of nutritional supplementation and resistance training as a more effective intervention to improve quality of life for people with sarcopenia. People with both malnutrition and sarcopenia have a higher mortality rate, so identifying people at risk of malnutrition and intervening early is extremely important to avoid sarcopenia and its associated problems. This article provides important information for dietary interventions in sarcopenia by summarizing the discoveries and developments of nutritional supplements such as protein, leucine, β-hydroxy-β-methylbutyric acid, vitamin D, vitamin C, vitamin E, omega-3 fatty acids, creatine, inorganic nitrate, probiotics, minerals, collagen peptides, and polyphenols in the management of sarcopenia.

KEYWORDS

sarcopenia, nutritional supplements, protein, leucine, β-hydroxy-β-methylbutyric acid, antioxidant, omega-3 fatty acids, creatine

1. Introduction

After the age of 50, muscle mass declines at a rate of 1%−2% per year in healthy adults, while muscle strength declines at a rate of 1.5% per year (1, 2). Sarcopenia was defined by the European Working Group on Sarcopenia in Older People (EWGSOP) in 2010 as "a syndrome characterized by progressive and generalized loss of skeletal muscle mass and strength with a risk of adverse outcomes such as physical disability, poor quality of life, and death" (3). In 2018, the EWGSOP updated the definition, and muscle strength and function are now put in front because the two are more important than muscle mass (4). Sarcopenia is a major public health concern because it affects 20% of people over the age of 70 and 50% of people over the age of 80 (5). Currently, 6%−19% of the global population over the age of 60 suffers from sarcopenia (6).

Sarcopenia should be suspected in patients who present with signs or symptoms such as falls, feeling weak, walking slowly, difficulty rising from a chair, weight loss, or muscle wasting, and EWGSOP2 recommends screening and evaluation starting with the Simple Five Item Scoring Questionnaire (SARC-F) (4). When low muscle strength is tested by grip strength or chair stand, it is considered very likely to have sarcopenia, because according to EWGSOP, the diagnosis of sarcopenia is "low muscle mass and low muscle function (strength or performance)," while muscle strength is the center of the diagnostic process (3). The Asian Working Group for Sarcopenia (AWGS) 2019 consensus defined "probable sarcopenia" as

low muscle strength or physical fitness, specifically used in primary care or community-based health promotion (7). In addition, low muscle mass can be measured by instruments such as DXA, BIA, CT, and MRI. The severity of sarcopenia can be determined by measuring physical performance, such as gait speed, SPPB, TUG, and 400 m walk (4). Ideally, a primary care physician should begin screening older adults at risk for sarcopenia, then diagnose them through the use of appropriate diagnostic tools, and begin treatment as early as possible. This will prevent delays in the diagnosis and management of sarcopenia. Common tools for screening and diagnosing sarcopenia and their respective advantages and disadvantages are shown in Table 1.

It has been reported that older residents at risk of malnutrition in Asian communities range from 16 to 73% (9), and the combined prevalence of moderate to high malnutrition risk among elderly people in Europe is 48.4% (10). In Latin America, two out of every five hospitalized patients are at risk of malnutrition (11). Malnutrition increases the risk of sarcopenia by two to three times, and people with sarcopenia who are undernourished have a higher mortality rate (12, 13). According to the AWGS consensus, older adults who present with low body mass index (BMI), unintentional weight loss, and low muscle mass or exhibit poor muscle strength at any time should be assessed for malnutrition, and those at risk of malnutrition should be rescreened every 3 months (9). Some evidence has shown that adequate intake of protein, vitamin D, antioxidant nutrients, and long-chain polyunsaturated fatty acids is beneficial for improving sarcopenia (14). Healthcare professionals are advised to provide nutritional counseling on dietary habits for older adults with sarcopenia and to collaborate with a dietitian to develop a diet and protein optimization plan for the patient (15). Therefore, identifying individuals at risk for malnutrition to provide early intervention is an important public health strategy to prevent the development of sarcopenia and related complications.

2. Nutritional intervention for sarcopenia

2.1. Protein, leucine, β-hydroxy-β-methylbutyric acid

2.1.1. Mechanisms

On the metabolic front, temporal fluctuations in muscle protein synthesis (MPS) and muscle protein breakdown (MPB) rates determine the net increase or decrease in skeletal muscle protein, which continuously remodels skeletal muscle mass. Protein degradation exceeding protein synthesis results in a negative nitrogen balance and triggers sarcopenia. Dietary protein or amino acid intake is the primary physiological stimulus for MPS (16), stimulating muscle protein synthesis, increasing muscle mass, and reducing muscle loss during bed rest and aging (17).

First, the ubiquitin–proteasome system (UPS) is the major pathway for cellular protein degradation, and studies have demonstrated that activating the UPS leads to increased protein degradation and finally results in sarcopenia (18, 19), while protein or amino acid nutritional support can effectively downregulate the levels of MuRF-1 and Atrogin-1 and ameliorate UPP-mediated sarcopenia (20, 21). Second, the oxidative response is one of the inducers of sarcopenia (22). Protein or amino acid nutritional support contributes to promoting Sirt1 expression, activating FoxO family proteins, enhancing the expression of SOD, and reducing the oxidative response (23). Third, enhanced autophagy evokes sarcopenia (24). Protein or amino acid nutritional support can enhance the activity of the PI3K/Akt/mTOR signaling pathway to suppress cell autophagy (25). There are some potential new mechanisms, including altering miRNA profiles and gut microbiota (26).

2.1.2. Clinical studies

2.1.2.1. Protein

To measure the effect of protein supplementation on muscle health, Hanach et al. analyzed 14 RCTs (27), with milk protein or protein-based dairy products for not <12 weeks as the intervention. The results showed that milk protein significantly increased limb muscle mass, although there was no effect on grip strength or leg muscle strength, and there was no conclusive evidence of an effect on physical activity. Kirwan et al. (28) analyzed 28 RCTs and showed that among older adults who performed resistance training, those who consumed higher protein increased lean limb mass and grip strength compared to controls who were supplemented with lower protein; however, without resistance training, there was no additional benefit from protein supplementation alone. In healthy older adults from Asia and other countries, the combination of protein supplementation and exercise significantly increased lower extremity strength compared to exercise alone or placebo, although no significant differences were found in upper extremity strength, muscle mass, or gait speed (29). Therefore, it is recommended to supplement protein in combination with resistance training to increase muscle mass and strength (30). Regarding the relationship between dietary protein intake and skeletal muscle mass, a cross-sectional analysis of 3,213 middle-aged and elderly residents in the mainland Chinese community found that participants who consumed more than 0.96 g/kg of protein per day had higher muscle mass than those who consumed no more than 0.96 g/kg of protein per day (31). In elderly subjects aged 70–85 years, those who consumed 1.5 g/(kg/day) of protein continuously for 12 weeks had higher skeletal muscle mass and mass index and higher gait speed, while the other two groups (0.8 and 1.2 g/kg/day of protein, respectively) did not differ significantly in terms of muscle mass and physical performance (32). A dose-dependent increase in whole-body net protein balance during recovery from resistance exercise in older healthy men randomly assigned to consume 0 g, 15 g, 30 g, or 45 g of milk protein concentrate suggests that the dose of protein consumed after exercise is a key factor in the magnitude of the muscle protein synthesis response (33). The World Health Organization and the U.S. The National Academy of Sciences currently recommends a protein daily allowance (RDA) of 0.8 g/kg/day for adults, but this value applies to all ages, regardless of gender, physical activity, or health status. Evidence from RCTs in elderly populations, as well as the protein requirements of elderly individuals measured using the indicator amino acid oxidation (IAAO) technique, suggests that this dose does not meet the physiological protein requirements of elderly individuals. The estimated average protein requirement

TABLE 1 Tools for screening and diagnosing (7, 8).

	Category	Disadvantages	Advantages
Screening tools	SARC-F questionnaire	Low to moderate sensitivity	Quick and easy to use
	Anthropometry	Measurement variability, lack of international standardization	Cheap, easy to perform
Diagnostic tools	Computed tomography (CT)	Gold standard for skeletal muscle mass	Expensive, high radiation
	Magnetic resonance imaging (MRI)	Cross-sectional analysis of muscle quantity and mass, no radiation	High cost, complex operation, time-consuming
	DXA (dual-energy X-ray absorptiometry)	Precise analysis of body composition, very low radiation exposure, operational	High cost
	Bioelectrical Impedance Analysis (BIA)	Accurate, inexpensive, simple, safe	Susceptible to fluid changes, etc.
	Ultrasound	inexpensive, simple, safe	Measurement accuracy to be verified

Anthropometry: Body mass index (BMI), mid-upper arm circumference (MUAC), calf circumference (CC), triceps skinfold (TSF).

(EAR) and RDA measured using IAAO technology were 0.94 and 1.24 g/kg/day in older men and 0.96 and 1.29 g/kg/day in older women, respectively (34, 35). According to the European Society of Clinical Nutrition and Metabolism (ESPEN), the diet of the elderly should provide at least 1.0–1.5 g protein/kg body weight/day, with 25–30 g protein allocated to each meal (36). However, patients with severe chronic kidney disease should limit their protein intake. In summary, most studies confirm that protein intake is positively correlated with muscle mass and strength and that higher protein intake has a positive effect on skeletal muscle health during aging. However, protein supplementation is not recommended as an independent intervention to improve muscle mass and strength. Protein supplementation only during resistance training can significantly improve grip strength and physical function, and the combination of the two can improve sarcopenia significantly more than resistance training alone (37–39). There is bias and heterogeneity in the evidence for protein supplementation on measures of muscle mass and the effects of strength and physical performance, and differences in the type and dose of protein supplementation, as well as variations in exercise regimen and duration, need to be taken into account when interpreting the results. More carefully designed large-scale randomized controlled trials exploring the effects of protein supplementation on these measures are needed in the future.

The quality and digestibility of proteins are distinguishing features between animal and plant proteins, with differences in amino acid content and absorption kinetics. Animal proteins such as meat, fish, and dairy products are consistently high-quality proteins, while plant proteins vary in quality depending on the sources, with soy protein being recognized as a high-quality plant protein. Therefore, with respect to the quality of protein, animal-derived protein may be more effective in maintaining muscle health. Regarding potential differences between animal and plant proteins affecting muscle health, a meta-analysis of 16 RCTs (51) showed that protein sources did not affect changes in absolute lean body mass or muscle strength; however, animal protein was more beneficial for percent lean body mass. It was shown in a retrospective study that men and women with higher animal protein intake had higher percentages of skeletal muscle mass regardless of physical activity, while the beneficial effects of plant protein were only shown in physically active adults

TABLE 2 Nutrients that may improve sarcopenia and recommended intake.

Nutrients	Recommended intake dose
Protein	1–1.2 g/kg/day for healthy elderly, 1.2–1.5 g/kg/day for the malnourished, or 25–30 g protein per meal (36)
Leucine	2.5–2.8 g per meal (36)
HMB	3 g/day (40)
Vitamin D	800–1,000 IU/day (41)
Vitamin E	400 IU/day (42)
Vitamin C	45 to 90 mg/day (43)
Magnesium	300 mg/day for men and 270 mg per day for women (44)
Selenium	25–75 μg/day (45)
Calcium	1,000–1,200 mg/day (46)
Probiotics	400 μg/day (47)
Inorganic nitrate	3.7 mg/kg/day (48)
Collagen	50 mL/day (49)
Polyphenols	>500 mg (50)

(52). In contradiction to these findings, a negative correlation between walking speed and relative animal protein intake and a positive correlation with relative plant protein intake have also been reported (53). Gazzani et al. (54) also supported the positive effect of plant proteins on physical performance and suggested that this could be related to other components of plant foods that affect muscle mass and strength, such as antioxidants. It is unclear how protein intake from different sources provides the best benefit for preventing sarcopenia, and further research is needed to refine protein dietary guidelines that promote muscle health.

2.1.2.2. Leucine

Amino acids are important raw materials for protein synthesis, and their homeostasis is essential for maintaining muscle health. Muscle protein synthesis is regulated at multiple physiological levels, including dietary protein digestion and amino acid absorption, visceral amino acid retention, postprandial insulin release, skeletal muscle tissue perfusion, muscle uptake of amino

acids, and intracellular signaling in myocytes (55). Therefore, some scholars have proposed that the anabolic potential of proteins correlates with amino acid composition, which is supported by the finding that plasma concentrations of leucine, isoleucine, and tryptophan are reduced in patients with sarcopenia (56). Synthesis by activating rapamycin complex 1 (mTORC1), a target that acts as a "switch" for the MPS process, which initiates translation in the intracellular signaling cascade (57). Thus, the "leucine trigger" hypothesis has been proposed, which predicts that the magnitude and rate of postprandial blood leucine increase may modulate the magnitude of the postprandial MPS response to protein intake. Sixteen of the 29 eligible studies provided sufficient evidence to support the hypothesis (58). Thus, leucine content may be a key factor in promoting the muscle protein synthesis response. The effect of 25 g of whey protein on maintaining skeletal muscle protein synthesis and improving muscle loss is similar to that of 10 g of milk with leucine in older adults (59). Compared to isonitrogenous protein drinks, protein drinks with higher concentrations of leucine are more beneficial for myogenic fibronectin synthesis (60). Leucine supplementation has been reported to have beneficial effects on body weight, body mass index, and lean body mass in older adults with a tendency toward sarcopenia, although the effects on muscle strength are inconclusive (61). Besides, according to a systematic review, protein supplements rich in leucine can improve markers of sarcopenia, regardless of physical activity, however, leucine supplementation alone and no exercise did not improve sarcopenia (62). Current evidence tends to recommend a higher intake of leucine in older adults to increase muscle mass. Considering the importance of leucine in muscle protein synthesis, leucine requirements in elderly individuals, measured using the indicator amino acid oxidation method, are more than twice the current recommendations, averaging 77.8 mg/(kg/day) for men and 78.2 mg/(kg/day) for women (63).

2.1.2.3. β-hydroxy-β-methylbutyric acid

β-hydroxy-β-methylbutyric acid (HMB) is a metabolite of leucine. The International Society of Sports Nutrition believes that HMB can reduce exercise-induced skeletal muscle damage and is most effective when consumed for two consecutive weeks before exercise, so athletes are recommended to take 38 mg per kg of body weight per day to promote their skeletal muscle growth and improve strength (64). The manufacturer usually recommends taking 3 g of HMB per day (40), the dose being equivalent to the intake of 60 g of leucine (65). However, if 60 g of leucine is consumed directly, the activity of rate-limiting enzymes for catabolism increases, and the oxidation of branched-chain amino acids increases, which can lead to depletion of valine and isoleucine in body fluids and ultimately an imbalance in the concentration of branched-chain amino acids, thus possibly having a negative impact on protein metabolism (66); however, there is wide heterogeneity in the conclusions drawn from published articles regarding the effects of HMB supplementation on muscle health and physical performance. Supporting research findings indicate that HMB intake promotes both upper and lower-extremity muscle strength in older adults (67). Supplementation with 3 g of HMB is most beneficial for improving strength and body composition in people over 65 years of age, especially when bed-rested and untrained (68). In the opposing study, Phillips et al. (69) stated through systematic evaluation and meta-analysis that the current evidence is insufficient to assess the effects of HMB supplementation on muscle function, as the evidence supports little and is inconsistent. In a randomized controlled trial carried out among 40 young adult men (70), the intervention group was supplemented with the leucine metabolites alpha-hydroxyisocaproic acid (α-HICA) and β-hydroxy-β-methylbutyric acid (HMB); as a result, supplementation with leucine metabolites did not enhance resistance training-induced changes in muscle thickness compared to placebo (71). In conclusion, more high-quality primary studies are needed in the future to investigate the effects of HMB in patients with sarcopenia, and the current evidence does not yet provide unambiguous support for recommending HMB supplementation to alleviate sarcopenia.

2.2. Vitamins

2.2.1. Vitamin D

2.2.1.1. Mechanisms

The vitamin D/VDR axis plays a key role in regulating biological processes central to sarcopenic muscle atrophy, such as proteolysis, mitochondrial function, cellular senescence, and adiposity (72). First, vitamin D deficiency appears to lead to increased muscle protein breakdown via the ubiquitin-proteasomal pathway (UPP) and autophagy and upregulation of AMPK and members of the renin-angiotensin system (73, 74). Second, permanent exit from the cell cycle (senescence) is a critical aging phenomenon, and the vitamin D/VDR axis has been shown to have regulatory control (75). Third, low vitamin D states may lead to impaired mitochondrial function (76), and active $1,25(OH)_2D3$ can increase oxygen consumption rates and fission/fusion dynamics (77, 78). Fourth, low vitamin D states may lead to increased adiposity in muscle (79), and those who are overweight have an increased risk of deficits in muscle mass and function (80).

2.2.1.2. Clinical studies

Vitamin D is a fat-soluble vitamin synthesized in the skin, 90% of which comes from UV exposure and 10% from the diet. Vitamin D deficiency is now considered a global public health problem, and elderly individuals are at greater risk of vitamin D deficiency due to poor intestinal absorption, reduced sun exposure, and chronic renal insufficiency. Lower 25-(OH)-VD levels are thought to be associated with adverse changes in muscle mass and physical function (81). Yang et al. (82) fed mice a vitamin D-deficient diet for 24 weeks and immobilized them to determine the extent of skeletal muscle atrophy. As a result, vitamin D deficiency accelerated the decrease in gastrocnemius muscle mass, muscle fiber cross-sectional area, and grip strength; moreover, vitamin D supplementation inhibited the decrease in grip strength. The team also performed a cross-sectional analysis of 4,139 older adults, and linear regression analysis showed that serum 25 hydroxyvitamin D and physical activity were linearly associated and interacted with timed running time and grip strength. However, in another study in which the control group took a placebo daily and the intervention group took 800 IU of vitamin D orally daily, no differences were found between the two groups in leg push-up strength, function,

or lean body mass after 1 year (83). According to the systematic reviews and meta-analyses, vitamin D supplementation alone did not improve muscle strength or SPPB scores, on the contrary, significantly decreased SPPB scores (84). When vitamin D was taken together with whey protein and leucine, the muscle mass of the limbs of patients with sarcopenia could be effectively increased even without physical exercise, and when combined with physical exercise, not only muscle mass increases but muscle strength and performance could also be significantly improved (85). However, we cannot be sure of the effectiveness of vitamin D supplementation alone, due to the presence of protein and amino acids. In summary, the exact role of vitamin D supplementation in the prevention and treatment of sarcopenia remains uncertain due to the high heterogeneity of studies and the conflicting results of RCTs.

2.2.2. Vitamin C and vitamin E

2.2.2.1. Mechanisms

With aging, the body's endogenous antioxidant defense system is impaired, and excessive accumulation of reactive oxygen species (ROS) in the body leads to oxidative muscle damage, which may be directly or indirectly involved in skeletal muscle atrophy (86). In addition, mitochondrial dysfunction occurs abnormally during muscle aging, which has been associated with aberrant ROS generation and oxidative damage (87). Antioxidant vitamins are thought to prevent oxidative stress and may be able to play a role in the treatment of sarcopenia. Therefore, whether antioxidant supplementation can improve age-related muscle mass and performance is becoming an issue of interest to researchers. Vitamins C and E are widely used antioxidant vitamins that have the ability to scavenge ROS and enhance cellular antioxidant capacity.

2.2.2.2. Related studies

Vitamin E is composed of two subgroups called tocopherols and tocotrienols. There are four isomers of tocopherols and tocotrienols (α, β, γ, and δ) depending on the number and location of the methyl groups, and their main dietary sources are vegetable oils, nuts, seeds, fish, shellfish, and vegetables (88). *In vitro*, studies have shown that alpha-tocopherol prevents myogenic cell atrophy and increases myotube survival (89), and the tocotrienol-rich fraction reverses the aging of myogenic cells by increasing the regenerative capacity of cells (90). Vitamin E contributes to the recovery of myogenic cell membranes and has a potential therapeutic effect on muscle cells (91), although further studies are needed to confirm the mechanisms involved. In a cross-sectional study, a significant positive association was found between increased dietary vitamin E intake and skeletal muscle mass index, bone mineral density status, and risk of total hip and hip fracture in middle-aged and older men and women, with effects ranging from 0.88 to 1.91% (92).

Vitamin C is the major water-soluble nonenzymatic antioxidant in plasma and tissues and must be obtained through dietary intake because it cannot be synthesized *in vivo*. A positive trend in quintiles of dietary vitamin C and lean body mass measurements suggests that dietary and circulating vitamin C is positively associated with skeletal muscle mass measurements in middle-aged and older men and women (93).

However, contrary studies have also been reported. In a systematic evaluation and meta-analysis, vitamins C and E did not promote muscle growth after strength training and may have diminished muscle hypertrophy over time (94). When young athletes were given vitamin C and E supplements, despite serum samples suggesting a reduction in oxidative stress in the body, participants' lower limb strength did not increase, and muscle damage could not be reduced (95). In summary, based on the existing evidence, there is not enough convincing evidence to support the use of vitamin E and vitamin C for the prevention and treatment of sarcopenia.

2.3. Omega-3 fatty acids

Omega-3 fatty acids (also known as n-3 fatty acids) are polyunsaturated fatty acids with many potential health benefits and are available in three main dietary forms: alpha-linolenic acid (ALA; 18:3n-3), eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3). ALA is considered an essential fatty acid because it cannot be synthesized in the human body and is found in nuts, seeds, canola oil, etc. EPA and DHA are mainly found in fish oil.

2.3.1. Mechanisms

Skeletal muscle atrophy involves an inflammatory phase that leads to cell death and tissue remodeling and activates endoplasmic reticulum stress (ERS) and autophagy (96, 97). Both EPA and DHA potentially attenuate ERS and autophagy in skeletal muscles undergoing atrophy by attenuating the increase in PERK and ATG14 expression (98). In addition, DHA promotes mitochondrial biogenesis and skeletal muscle fiber remodeling (99) and delays muscle wasting by stimulating intermediate oxidative stress and inhibiting proteasomal degradation of muscle proteins (100).

2.3.2. Related studies

It has been proposed that elevated plasma levels of proinflammatory cytokines affect muscle catabolic and anabolic signaling pathways and thus may play a key role in the development and progression of sarcopenia, with data showing significantly elevated levels of IL-6 and TNFα in elderly Chinese individuals with sarcopenia (101). Therefore, reducing chronic inflammation associated with aging is emerging as a potential therapeutic target for sarcopenia, and NSAIDs may not be recommended for the treatment of sarcopenia due to the high risk of adverse events that may occur with their use in elderly individuals. Increasing evidence indicates that omega-3 polyunsaturated fatty acids reduce the expression of inflammatory genes and have anti-inflammatory activity (102), particularly eicosapentaenoic acid, docosahexaenoic acid, and alpha-linolenic acid. It was concluded from a systematic evaluation and meta-analysis that omega-3 fatty acid supplementation promotes lean body mass, skeletal muscle mass, and isometric contraction maximal muscle strength in the quadriceps (103). Dietary omega-3 fatty acid levels are negatively associated with sarcopenia (104), and more than 2 g/day of omega-3 fatty acids may increase muscle mass and improve

walking speed, especially for those with sarcopenia who have been receiving the intervention for more than 6 months (105). However, linear regression analysis concluded that there was no association between plasma omega-3 levels and grip strength in older adults (106). When cancer patients were supplemented with omega-3 fatty acids, their muscle maintenance, quality of life, and body weight were not improved (107). According to expert opinions (108), doses of 3,000 mg/day DHA plus EPA or more (with preferably more than 800 mg/day EPA) may be required for positive physical performance in older adults (109, 110), because lower doses have no significant effects on muscle strength (111). In conclusion, omega-3 fatty acids may improve sarcopenia, but well-designed, large prospective cohort studies and randomized controlled trials are needed to confirm these findings.

2.4. Creatine

Creatine is a natural nonprotein amino acid compound. Approximately half of the daily creatine requirement comes from the diet, mainly in red meat and seafood (112), and the other half is synthesized endogenously in the kidneys and liver (113). Creatine is mainly stored in muscle (95%), ∼2/3 is in the form of PCr, and the rest is free creatine. Approximately 1%−2% of intramuscular creatine is degraded to creatinine and excreted in the urine each day (114, 115); therefore, the body needs to replenish ∼1–3 g of creatine per day to maintain normal creatine stores and to obtain the free energy provided by catabolism, depending on muscle mass (116).

2.4.1. Mechanisms

The energy produced by phosphocreatine (PCr) degradation is used to resynthesize ADP and Pi back into ATP to maintain cell function. Increasing PCr and creatine in muscles provides energy reserves to meet anaerobic energy needs, providing a critical source of energy, especially during ischemia, injury, and/or response to impairment (117, 118). Creatine has been shown to activate signaling pathways in the muscle protein synthesis pathway (119), and creatine also protects against mitochondrial damage caused by oxidation, which may reduce inflammation and muscle damage (120, 121).

2.4.2. Clinical studies

After examining the effects of different creatine dosing strategies (lower: 5 g/day, higher: >5 g/day) and the presence or absence of a creatine loading phase (20 g/day for 5–7 days) on lean tissue mass and strength, overall, creatine increased lean tissue mass and strength, but when studies involving a creatine loading phase were excluded from the analysis, creatine had no greater benefit on muscle mass and strength compared to placebo and was effective only during the resistance training phase (122). In another study, creatine supplementation significantly increased upper extremity strength but had no effect on lower extremity strength or muscle mass. However, when resistance training was continued for at least 24 weeks, a significant increase was found in upper and lower extremity muscle strength among older females (123). Overall, creatine intake during resistance training in older adults may increase lean tissue mass, as well as muscle strength in the upper and lower extremities (124). Therefore, it is recommended that older adults supplement creatine concurrently with resistance training. Creatine supplementation appears to enhance the muscular adaptive response to training by increasing the ability to exercise at high intensities and enhancing postexercise recovery and adaptation (125). Differences in creatine dose and frequency of intake during resistance training need to be considered when interpreting the heterogeneity between these studies (126).

2.5. Inorganic nitrate

The health benefits of a diet rich in vegetables are partly explained by their high nitrate content, which is an important biologically active cardioprotective component of vegetables due to its effects on endogenous nitric oxide and vascular health (127). Approximately 80% of total dietary nitrate intake comes from vegetables, with leafy greens and beet being the most abundant and the rest from fruits and meat (128).

Skeletal muscle tissue is the largest reservoir of nitrate in the body and one of the main sites of nitrate and nitrite metabolism (129), which is sensitive to dietary nitrate intake, contributing to nitric oxide production during exercise, and it enhances human muscle contraction by increasing the free intracellular calcium concentration and the calcium sensitivity of myofilaments themselves (130, 131). A cross-sectional analysis revealed that higher nitrate intake (mean 31.2 mg/day) is associated with stronger grip strength and faster timed runs (132). Researchers evaluated participants' habitual dietary intake over 12 years in a cohort study, and individuals with the highest nitrate intake (mean 91 mg/day) had stronger knee extension and faster timed starts, and the results were unaffected by physical activity (133). In randomized controlled trials, nitrate is given almost in the form of concentrated beetroot as an acute dose ranging from 6.4 to 15.9 mmol, and the results show that NO_3^- intake significantly increases muscle strength, with an average increase of ∼5% (134). Its potential benefits on muscle strength and endurance are not affected by dose, frequency of intake, level of training, muscle group, or type of contraction (135). It may improve grip strength in older adults by accelerating muscle oxygenation and muscle strength recovery after exercise (136). Most of the current research suggests that a nitrate-rich diet has potential benefits for muscle strength and physical function in older adults, but due to the lack of research, more evidence is needed to validate this claim.

2.6. Probiotics, prebiotics, synbiotics

Probiotics are beneficial bacteria that are mainly found in our digestive system. Prebiotics are mainly oligosaccharides that promote the growth and proliferation of beneficial bacteria in the body but are not digested and absorbed by the host (137). Preparations that mix probiotics and prebiotics are called synbiotics (138), and the benefits of both are unified.

2.6.1. Mechanisms

Changes in the structure of the intestinal flora are closely related to human health and disease. The major phyla of the healthy intestinal microbiota are the thick-walled phylum, the phylum Bacteroidetes, the phylum Actinomycetes, and to a lesser extent, the phylum Wolbachia and the phylum Aspergillus. In weak and cachectic humans, these beneficial bacteria are reduced, while an increase in opportunistic pathogens of Enterobacteriaceae occurs (139). Probiotics promote the production of metabolites such as short-chain fatty acids (SCFAs), secondary bile acids (BA), and some amino acids that regulate homeostasis in skeletal muscle by improving insulin sensitivity (140, 141). In addition, alterations in the ecosystem composition of the gut microbiota, such as reduced production of beneficial metabolites (e.g., SCFA) in the intestinal lumen, lead to intestinal leakage and bacterial endotoxins such as lipopolysaccharides (LPS) entering the peripheral blood (142), producing systemic inflammation associated with aging and muscle wasting (143, 144). Probiotics can limit inflammation and oxygen stress (145).

2.6.2. Related studies

The experimental model of germ-free mice provides valuable evidence for the potential role of the microbiota in controlling muscle mass and function. Compared to conventional mice, germ-free mice, and antibiotic-treated mice, their muscle mass and strength are decreased (146–148). Interestingly, this change in muscle mass and function can be restored by transplantation of microbiota or under natural conditions (146, 147). The elderly were divided into high-functioning (HF) and low-functioning (LF) groups based on physical function, stool samples from both groups were transferred to germ-free mice, and grip strength was significantly increased in mice with HF compared to mice with LF (149). In mouse and human models, reduced intestinal permeability usually coincides with improved muscle mass or strength (150). The use of probiotics, prebiotics, and synbiotics may thus reduce muscle mass loss by stimulating the growth of the bacterial flora and restoring the balance of the gut microbiome, ultimately resulting in a more beneficial metabolite profile and lower intestinal permeability. COPD patients with sarcopenia who were continuously supplemented with a multistrain probiotic for 16 weeks showed reduced markers of intestinal permeability and neuromuscular junction degeneration in plasma, along with improved grip strength, gait speed, and SPPB scores compared to the placebo group (151). However, the causal relationship between microbiota and muscle health remains uncertain due to the lack of targeted studies and the effects of a large number of covariates (including diet, exercise, polydipsia, and multiple drugs) on microbiota composition and function (152). In addition, specific strains that optimize muscle mass and function are not yet available due to the scarcity of human studies and the difficulty of accurate measurements. Future studies should be conducted in humans and should focus on the effects of different bacterial genera and strains on microbiome balance, metabolite profiles, gut function, and muscle mass in sarcopenia.

2.7. Magnesium, selenium, calcium, and other minerals

Growing evidence shows that low micronutrient intake is associated with an increased risk of sarcopenia (153). It has been shown from systematic evaluations that patients with sarcopenia have lower intakes of calcium, magnesium, sodium and selenium than older adults with healthy muscles (154), and magnesium, selenium and calcium appear to be the most promising minerals for the prevention or treatment of sarcopenia (155).

Magnesium is involved in numerous physiological processes as a cofactor in many enzymatic reactions, and it also plays an important role in maintaining muscle mass and protecting muscle tissue from oxidative damage (156, 157). Mg^{2+} supplementation in aged mice induces myogenic differentiation, promotes protein synthesis, provides protection against the loss of muscle regeneration potential and muscle mass during aging, significantly promotes muscle regeneration, and preserves muscle mass and strength (158). The study suggests that intramuscular ionized magnesium is negatively correlated with age and positively correlated with the strength of knee extension in females. This may be because females have chronic underlying magnesium deficiency and therefore have significantly lower intramuscular ionized magnesium than males (159). In a cross-sectional study involving 2,570 women aged 18–79 years (156), a positive association between dietary magnesium intake and skeletal muscle mass and explosive leg strength index was observed, and data from another prospective cohort study suggested that higher magnesium intake is associated with greater grip strength and higher skeletal muscle mass (157). In follow-up surveys over 5 years, increased magnesium intake was associated with increased SPPB scores in older women, but no such association was observed in men (160). Higher intake of magnesium has been shown to be positively correlated with appendicular muscle mass and change in appendicular muscle mass in a longitudinal study (161), and positive associations between magnesium intake and grip strength have been shown in a cross-sectional study (162). There is some consistency in the current studies of magnesium's ability to improve sarcopenia, suggesting that magnesium supplementation may slow age-related skeletal muscle mass loss, although the evidence is mainly observational and cross-sectional studies.

Selenium is one of the essential trace elements, and it has been reported that patients with selenium deficiency develop skeletal muscle disease, manifested by muscle pain, fatigue, proximal limb weakness, and elevated serum creatine kinase (163). Although dietary supplements of selenium alone or in combination with vitamins are being widely used, the effects of selenium on muscle performance have not been adequately studied. Experiments conducted in mice show that selenium supplementation increases calcium release from the sarcoplasmic reticulum, thereby improving skeletal muscle performance, and that increased expression of selenoprotein N in muscle enhances oxidative stress tolerance (164). Selenium concentrations were found to be negatively associated with restricted physical function in a cross-sectional study, with a reduced incidence of physical frailty when baseline selenium levels were doubled (165). In the only clinical randomized controlled study, combined vitamin E,

vitamin C, zinc, and selenium supplementation for 17 weeks improved maximal voluntary contraction and endurance limit time in the quadriceps muscle by reducing oxidative stress and enhancing antioxidant defense (166). Although selenium intake is low in elderly individuals and correlated with poorer skeletal muscle function, prospective analysis indicates no significant effect of selenium intake on skeletal muscle function (167). Nevertheless, the daily dietary intake of selenium is 20–75 μg for adults according to the EU recommendations (45). Since most of the evidence is from observational studies, we are not yet able to conclusively determine the effect of selenium supplementation in patients with sarcopenia; thus, large randomized controlled trials are required in the future to demonstrate this.

In the cross-sectional analysis, daily calcium intake was negatively correlated with overall fat percentage and positively correlated with extremity bone mass. After adjusting for age, sex, BMI, total energy intake, and lifestyle factors, daily calcium intake was significantly lower in patients with sarcopenia than in those without sarcopenia (168). However, 6 months of calcium supplementation does not have a significant effect on skeletal muscle strength and serum testosterone in young adult men, as found in one randomized controlled trial (169). There is a lack of studies on the effect of calcium on patients with sarcopenia.

2.8. Collagen and collagen peptides

Collagen accounts for one-third of the total protein in the human body, is the most abundant form of structural protein in the body, and contributes about 65%−80% of tendon dry weight (170). Extramyocellular connective tissue transmits contractility to tendons and bone, and collagen is a core structural component of extracellular connective tissue and is therefore essential for the strength, regulation, and regeneration of this tissue (171). Dietary collagens, such as collagen peptides or gelatin, are most commonly extracted from the skin, bones, or scales of pigs, cattle, and other poultry (172), and because they contain large amounts of glycine and proline and hydroxyproline, similar to the amino acid distribution of muscle connective tissue, it has been proposed that increasing their intake may help to stimulate muscle connective tissue synthesis to the greatest extent (173), thereby increasing muscle mass and strength and improving sarcopenia possibly.

2.8.1. Mechanisms

Consumption of proline-rich and glycine-rich collagen may be more suitable than high-quality protein sources such as casein or whey protein (providing only 6% proline and 2% glycine) to provide specific amino acid precursors required to support *de novo* synthesis of connective tissue proteins since the amount of glycine and proline provided in the usual diet is insufficient to provide metabolism and promote increased rates of tissue collagen synthesis (174, 175). In an *in vitro* model, tendons in growth mediums containing proline and ascorbic acid showed increased collagen content and improved mechanical properties (176). In rats, a glycine-rich diet made the Achilles tendinitis model more resistant to maximum tolerated loads (177). In addition, peptides produced by collagen hydrolysis, which are easily absorbed in the digestive tract before entering the circulation (178), can enhance fibroblast elastin synthesis, while inhibiting elastin degradation and promoting fibroblast proliferation (179), and thus may enhance connective tissue remodeling in muscle.

2.8.2. Clinical studies

Regarding the effect of collagen supplementation on body composition, Zdzieblik et al. (180) showed that elderly men with sarcopenia exercised three times a week and ingested 15 g of collagen peptide per day for 12 weeks, and their changes in body composition were very significant, with a mean increase in fat-free mass of 4.2 kg compared to only 2.9 kg in the placebo group. The same test in young, healthy men resulted in a mean increase in fat-free mass of 2.6 kg in the collagen peptide group and only 0.7 kg in the placebo-supplemented group (181). In premenopausal women, it was also found that the combination of resistance training with collagen supplementation significantly increased fat-free mass and increased hand grip strength. The above studies showed that collagen peptide supplementation was effective in improving muscle mass and strength while resisting resistance exercise. Regarding the effect of collagen on muscle protein synthesis, Oikawa et al. (182) supplemented 30 g of whey protein or collagen peptide twice a day in older adults who lacked physical activity and low-energy status, and only the whey protein group enhanced fat-free mass and muscle protein synthesis in the lower extremities during return to activity. Two other studies have similarly observed increased muscle protein synthesis with whey protein compared to collagen supplementation (183, 184). This suggests that collagen has little anabolic potential compared to isonitrogenous higher-quality protein sources. According to systematic reviews and meta-analyses, collagen supplements are most beneficial in reducing joint pain and improving joint function, with some improvement in body composition, strength, and muscle recovery (170). In conclusion, collagen supplementation with resistance exercise can increase muscle mass and strength, but there is insufficient evidence that collagen is more effective in improving sarcopenia than traditional high-quality protein sources such as casein or whey protein.

2.9. Polyphenols

Polyphenols are a range of plant compounds with antioxidant and anti-inflammatory properties containing one or more phenolic rings attached to hydroxyl groups (185). They are divided into four classes: phenolic acids, flavonoids, stilbenes, and lignans (186) and are particularly abundant in fruits, vegetables, coffee, tea, cocoa, vanilla, and spices (187). Because there are a wide variety of polyphenols available and there are many factors that can alter their concentration in food, it is difficult to establish reference composition tables (188).

2.9.1. Mechanisms

The effects of polyphenolic compounds in dystrophia are mainly through the inhibition of E3 ubiquitin ligases

and upstream regulators in inflammation, oxidative stress, and mitochondrial damage (189, 190). It also increases protein synthesis by effectively activating the Akt/mTOR pathway (191). Moreover, PPs modulated the expression of miRNAs, IGF-1 signaling pathway, follistatin, mitochondrial biogenesis, and myogenic differentiation factors involved in myogenesis (192).

2.9.2. Related studies

Resveratrol (RSV) is a natural polyphenol. In animal experiments, high doses of RSV (400 mg/kg/day) have been reported to attenuate muscle fiber atrophy following hindlimb suspension in rodents (193). Lower doses of RSV (5 mg/kg/day) still promoted skeletal muscle hypertrophy and reduced exercise-induced muscle necrosis in wild-type mice (194). In clinical studies, elderly subjects were supplemented with 500 mg/day resveratrol during exercise, and muscle mitochondrial density and muscle fatigue resistance were higher in elderly subjects compared with placebo-supplemented groups (195). Resveratrol at 1,000 mg/day increased the 6-min walk distance by 33.1 m in older adults, which was higher than the mean walking distance in the 500 mg/day group (196). Patients with chronic kidney disease received 500 mg resveratrol and 500 mg curcumin orally daily, and muscle mass and bone mass increased significantly after 12 weeks. However, no improvement in walking ability with resveratrol was observed in elderly subjects with peripheral arterial disease (197), mitochondrial function in skeletal muscle was not improved and lean body mass was decreased in COPD patients receiving 150 mg/day resveratrol (198). To determine the effects of polyphenols on muscle, multiple systematic reviews and meta-analyses have assessed the effectiveness of polyphenols on muscle pain and muscle recovery after exercise in healthy adults, and the results have shown that consumption of polyphenol-rich foods, juices, and concentrates accelerates the recovery of muscle function and reduces muscle soreness at doses ranging from 150 to 1,500 mg/day (199–201). A meta-analysis suggests that polyphenol supplementation is unlikely to enhance exercise-induced changes in body composition or performance, and that only isoflavones may increase lean body mass in postmenopausal women (202), and another meta-analysis suggests that short-term polyphenols intake, although attenuating the inflammatory response after exercise, does not affect the anabolic response to protein and exercise in healthy elderly men (203). In summary, polyphenol supplementation is believed to reduce muscle pain and accelerate the recovery of muscle function after exercise, but the effect on body composition and physical performance in patients with sarcopenia is inconclusive and remains to be explored.

3. Conclusions

Clinicians or health care providers need to screen older adults at risk for sarcopenia, especially those with comorbid malnutrition, and use appropriate diagnostic tools to make the diagnosis. Related professionals should then provide resistance training and diet and protein optimization programs to patients with diagnosed sarcopenia (15). This article summarizes the research progress of nutritional supplements in the improvement of sarcopenia, including the possible cellular and molecular mechanisms involved, so as to provide a reference for medical staff and researchers. The currently acceptable recommended intakes for each nutrient are shown in Table 2.

In the future, patients may benefit from complex hybrid nutritional supplements, as well as the development of nutrigenomics and metabolomics (204), so that nutritional interventions provided are tailored to an individual's nutritional and metabolic status. In addition, when the molecular mechanisms of muscle targets are well studied, they may play a key role in developing targeted treatment and prevention strategies.

Author contributions

SLiu contributed to drafting the paper. LZ had primary responsibility for final content. LZ and SLi revised the final draft of the manuscript. All authors read and approved the final manuscript.

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Conflict of interest

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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"We want more": perspectives of sarcopenic older women on the feasibility of high-intensity progressive resistance exercises and a whey-protein nutrition intervention

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This qualitative study is nested within a 12-week pilot randomized-controlled, two-arm trial involving high-intensity progressive resistance training (PRT) or PRT with a multi-nutrient, whey-protein supplementation (PRT+WP) in sarcopenic older adults (trial registration no: TCTR20230703001). The aim was to investigate sarcopenic participants' perceptions and barriers to this multi-modal intervention strategy that may accelerate "real-world" implementation. Eighteen older adults (one man) with possible sarcopenia were invited to join the study, of whom 16 women were randomized to a thrice-weekly PRT ($n = 8$) program (80% of 1-repetitive maximum, six resistance band exercises) only or PRT plus daily weekday milk-based WP supplementation (PRT+WP, $n = 8$). Muscle strength (handgrip and 5-times sit-to-stand), mass (dual-energy X-ray absorptiometry), performance (Short Physical Performance Battery and stair ascent-descent), and nutrition status (Mini Nutritional Assessment) were assessed for changes. We randomly selected eight women for the semi-structured interview. Post-intervention, eight (50%) women were sarcopenia-free, six (38%) remained in possible sarcopenia, one (6%) improved to sarcopenia, and one (6%) deteriorated from possible to severe sarcopenia. There were no significant between-group differences, but significant within-group improvements ($p < 0.05$) were detected for handgrip strength (PRT+WP 5.0 kg, $d = 0.93$; PRT 6.1 kg, $d = 0.55$), 5-times sit-to-stand time (PRT 2.0 s, $d = 1.04$), nutrition score (PRT+WP 3.44, $d = 0.52$; PRT 1.80, $d = 0.44$), and stair ascent time (PRT+WP 0.97 s, $d = 0.77$; PRT 0.75 s, $d = 0.97$). Our thematic analyses identified four main themes, namely, (1) perceived benefits, (2) sustaining behavior changes, (3) challenges in participating, and (4) improved wellbeing. Participants expressed how they initially were skeptical and doubted that they could complete the exercises or tolerate the milk-based WP supplements. However, they reported positive experiences and benefits felt from strength gains, increased confidence, and better physical abilities. Participants were surprised by the zero adverse effects of WP supplements. The women wanted more nutritional information and structured, guided exercise programs and suggested a community-based implementation. In conclusion, our findings showed PRT was well received and may support reduced risks of sarcopenia. No added benefits were seen with the addition of WP supplementation, but a larger sample is required to address this question. Overall, older (previously sarcopenic)

Malay women indicated that they want more multi-modal programs embedded in their community.

KEYWORDS

sarcopenia, older adults, resistance exercises, whey-protein, qualitative, pilot, randomized-trial

1. Introduction

Sarcopenia is an age-related, progressive, and generalized skeletal muscle disorder involving a loss of muscle mass, strength, and function that is associated with increased adverse outcomes including falls, frailty, fractures, and premature mortality in older adults (1). The overall prevalence of sarcopenia is estimated to be 10% worldwide in those aged at least 60 years and above, with some reports of a higher prevalence among Asians than non-Asian countries (2). Sarcopenia prevalence is further confounded by the different cut-points adopted by several key working groups such as the European Working Group on Sarcopenia in Older People, the Asian Working Group for Sarcopenia, and the International Working Group on Sarcopenia that may be region-specific in their application (2). Research in Malaysia on sarcopenia is still limited, but one study among 393 adults aged 60 years and over reported an overall prevalence of 33.6% (3). The prevalence of sarcopenia (and its components) is related to exorbitant healthcare costs (4), and thus the maintenance of muscle mass, strength, and function is critical to avoid the financial burden related to home-assisted living and institutionalized care in older people (5). With no known pharmaceuticals available to treat sarcopenia (6, 7), strategies to increase muscle mass, strength, and function focus on modifiable lifestyle behaviors and practices that have been shown to be effective.

Adequate nutrition, such as high protein intake, sufficient calories, adequate omega n-3 fatty acid intakes, and regular exercise, is widely recommended for people diagnosed with sarcopenia (8). More specifically, current guidelines for the treatment and prevention of sarcopenia recommend muscle strengthening or progressive resistance training (PRT) along with an adequate intake of protein and sufficient vitamin D (9–12). In sarcopenic older adults, PRT exercises are safe and effective to improve muscle health, and the addition of protein with PRT may provide some additional, albeit modest, benefits to muscle mass and strength (13, 14). Despite these benefits, adherence to PRT among community-dwelling older adults remains low (<10–15%) (15). Older people's uptake and engagement with such programs can be influenced by a range of behavioral factors, such as motivation and personal beliefs, as well as environmental factors, including the availability of public transport, cost, and the location and type of exercise venues (16).

Despite the positive responses from previous studies evaluating the effectiveness of exercise and/or nutritional intervention on sarcopenia outcomes (13, 14), there has been little research performed in Malaysia. A large proportion of older adults in Malaysia are physically inactive, with 30% reporting not engaging in any regular activity (17). The Malaysian community comprises different ethnicities, body sizes and compositions, food cultures, and physical activity habits compared to other populations (18, 19). To date, no studies have explored the beliefs and perceptions of older Malaysian adults with or at risk of sarcopenia about participating in a multi-modal lifestyle intervention incorporating high-intensity PRT with a whey-protein-based nutrition supplemental drink (WP). This is important to inform our understanding of the behaviors (motivation and barriers) and decision-making on sarcopenia in this population to inform future initiatives to prevent and treat this disease and support sustainable behavior change. Therefore, this pilot study aimed to identify the key facilitators and barriers for older Malaysian adults with sarcopenia to participate in a 12-week PRT program with or without the consumption of a WP nutritional supplement.

2. Materials and methods

2.1. Study design

This is a qualitative study nested within a 12-week randomized-controlled, two-arm trial involving PRT alone or combined PRT+WP supplementation. This research was approved by the Human Research Ethics Committee of Universiti Sains Malaysia (USM/JEPeM/18090405).

2.2. Participants

We reached older Malaysian adults through online advertisements and community-focused areas such as mosques, community halls, and private health facilities. Using the Asian Working Group for Sarcopenia 2019 (AWGS2) (20) guidelines, we initially screened for possible sarcopenia and included participants with a handgrip strength (HG) of <28 kg (men) or <18 kg (women) using their dominant arm and/or a five-times sit-to-stand time (5-STS) of 12 s and more. HG test was conducted using a hand-held dynamometer (Jamar, USA), where the participants were seated and their arms bent at 90° at the elbow. Participants were given verbal encouragement during each test and were instructed to squeeze as hard as they could and alternate arms to complete the test three times per arm. The highest value (in kg) obtained was used as the maximal HG strength. Participants who were identified as having possible sarcopenia, physically inactive (did not achieve 150 min/week of physical activity), and did not participate in any structured exercise programs in the past 6 months were invited to join the study. Participants

who provided informed written consent later completed the rest of the assessments at the Universiti Sains Malaysia, Health Campus, Kelantan.

2.3. Physical measures

The following measures were assessed at baseline and post-intervention: Height measurements were conducted three times using a stadiometer (SECA, Germany), and participants' weight was measured using a digital weighing scale (Omron, USA). Appendicular skeletal muscle mass (ASM, kg/m^2) was assessed from a total body dual-energy X-ray absorptiometry scan (DXA, Discovery A, Hologic, USA), with low ASM classified as <7.0 kg/m^2 for men and <5.4 kg/m^2 for women (20). Physical performance was assessed using the Short Physical Performance Battery (SPPB) that includes balance (side-by-side, semi-tandem, and tandem), 5-STS, and a 4-m gait assessment (21), with impaired physical performance classified as a SPPB score of ≤9. Sarcopenia classifications followed the possible sarcopenia (low grip strength and/or slow 5-STS time), sarcopenia (possible sarcopenia or low SPPB plus low ASM), and severe sarcopenia (presence of all three low measures) definitions from AWGS2 (20). Participants also completed the Mini Nutritional Assessment (MNA) that includes measurements of left-side mid-arm and calf circumferences (22). Additionally, we assessed stair ascent and descent time using a standardized set of 10 steps, whereby participants were asked to walk as quickly up or down the stairs as possible, with the time recorded to the nearest 0.1 s (23).

2.4. Intervention

A total of 16 participants (women) were randomized to the PRT+WP ($n = 8$) or PRT alone ($n = 8$) groups. A research assistant used simple computer randomization to allocate the participants into their groups. Participants in the PRT+WP group had a daily weekday serving of 100% whey protein concentrate (Enprovis Plus, Aegisu Sdn. Bhd.) that consists of 237 kcal, 15.1 g protein, 250 mg calcium, 3.3 mg iron, 4.0 μg vitamin D, and other micronutrients that cover 14–93% of the daily recommended nutrient intake for those aged 60 years and above. The full nutrition composition is provided in Supplementary material 1. The supplement was consumed as part of their morning breakfast on non-exercise days or within a 30-min window after their exercise sessions.

All participants were prescribed a high-intensity PRT program that could be conducted at home thrice a week. Prior to commencing PRT, a qualified Master of Science in Exercise Science graduate assessed baseline muscle strength and taught proper exercise techniques and warm-up and cool-down activities to all participants. Participants had their blood pressure and heart rate checked using an automated blood pressure device (Omron, Japan) after sitting quietly for about 3 min to rule out uncontrolled hypertension. Under supervision, participants practiced the exercise movements without any resistance bands twice a week for 2 weeks before the intervention period started. There were six resistance band exercises, namely, (i) squats, (ii) gluteal kickbacks, (iii) seated leg extensions, (iv) standing chest press, (v) standing diagonal pull apart, and (vi) seated row. To assess 1-RM, participants were given a resistance band to complete 10–12 repetitions of each exercise without losing their form or technique. If participants could complete 12 repetitions easily, a thicker (higher) resistance band was provided, and the same exercise was conducted after a 2–3 min rest until the suitable resistance band was identified as the participant's 1-RM load. If participants struggle to complete eight reps adequately, they will be given a thinner (lower) resistance band. As the same resistance band can provide varied tension depending on the tautness, participants were taught to hold their resistance bands in a straight line, with no slacking or excess pull, at the starting position of each exercise. For the first 2 weeks of familiarization, participants used light resistance bands, performing two sets of 10–15 repetitions for each of the six exercises. Exercises increased progressively, with adjustments occurring at weeks 2, 4, and 8. At each progression check, only one component was increased, such as repetitions or adding an additional set. If participants reached three sets of 10 reps with the current resistance band, the resistance band was changed to the next higher resistance band with a reassessment of the number of reps and/or sets to be conducted. Participants achieved and maintained their individualized equivalent of 75–80% of 1-RM throughout the rest of the intervention period and progressed to three sets of 8–10 repetitions for about 4 weeks before the intervention concluded. The resistance bands, length 2.0 m × width 150 mm (Exercise Band, Top Glove, Malaysia), have a variable thickness of 0.15–0.45 mm (increments of 0.05 mm), and the maximal band thickness used by participants was 0.20 mm, or the equivalent of 4.3–4.7 kgf at 300% extension (manufacturer's information). All exercise prescriptions and progressions were conducted in person and then monitored through weekly inquiries by the research team that checked on the completion of exercise and supplement intake based on the participants' self-report for that week. During the weekly calls, participants were also asked to report any adverse events related to the prescribed exercise or nutritional supplement.

2.5. Semi-structured interviews

Upon completing the 12-week intervention, using a purposive sampling method, a total of 8 of the 16 participants participated in a semi-structured interview, achieving the data saturation concept. Data saturation was reached when the information from participants no longer offered new insights and views that others had not mentioned (24). Each interview lasted approximately 30–40 min using an interview guide (Supplementary material 2). Interviews were conducted over the phone at the participant's convenience as this was during the COVID-19 pandemic lockdown in Malaysia and face-to-face interviews were not possible. Interviews were recorded in a local Malay dialect, translated to English, and transcribed by the same trained research assistant. There were no pilots or repeat interviews. Our qualitative researcher counter-checked the translation and transcription and the field notes made during the interviews. The NVivo software, version 12 (QSR International, Australia), was used by the

researcher to organize, explore, integrate, and finally interpret the data. Thematic analysis using the six phases by Braun and Clarke (25) provided rigorous and trustworthy data analysis and interpretation. Data were presented according to emerging themes that were discussed and agreed upon by two researchers. Qualitative reports were guided by the consolidated criteria for reporting qualitative research (COREQ) checklist (26).

2.6. Statistical analyses

Statistical analyses of the quantitative measures were conducted using SPSS Statistics for Windows, version 26 (Armonk, NY: IBM Corp.). The data met the assumption of normality, and baseline group differences were assessed using an independent *t*-test. Mean group differences for the change (post-intervention minus baseline values) were generated and compared using an independent *t*-test. Within groups, changes were assessed using a paired *t*-test, and assumptions were checked and matched for the inferential statistical analyses. Observed effect sizes of paired samples (within-group) were calculated using unbiased Cohen's *d* (post-intervention means minus baseline means, then divided by pooled standard deviations with correction), with an interpretation of the magnitude of *d* based on the following: 0.20 "small effect," 0.50 "medium effect," and 0.8 "large effect" (27). For the effect size of the net differences (between groups), *d* was calculated using the mean changes in PRT+WP minus the mean changes in PRT and divided by the pooled standard deviations of the respective means. Due to our modest sample size, the results and findings from the statistical analyses should be interpreted with caution. Statistical significance was set at a *p*-value of <0.05.

3. Results

3.1. Study participants, attrition, and adherence

A total of 320 older adults were screened for this study, of whom 18 were identified as having possible sarcopenia (one man). Two participants (husband and wife) withdrew from the PRT group after citing personal concerns about attending measures and exercise intervention sessions. Notably, 16 women were randomly allocated to the PRT+WP ($n = 8$) or PRT ($n = 8$) groups. The recruitment and randomization process is displayed in Figure 1 based on the CONSORT extension to randomized pilot study guidelines (28).

Participants were community-dwelling, Malay women, aged (mean ± SD) 66.0 ± 3.1 years (range 61–72). Characteristics of the participants by PRT+WP and PRT group are displayed in Table 1. There were no significant differences between groups at baseline for all the measures, indicating randomization was achieved.

Overall, 16 (100%) participants completed the intervention and the post-intervention measurements. We recorded that the participants had 90% adherence to the prescribed exercises and supplement intake throughout the intervention period. There were no reported adverse events from either the exercise or nutrition supplement.

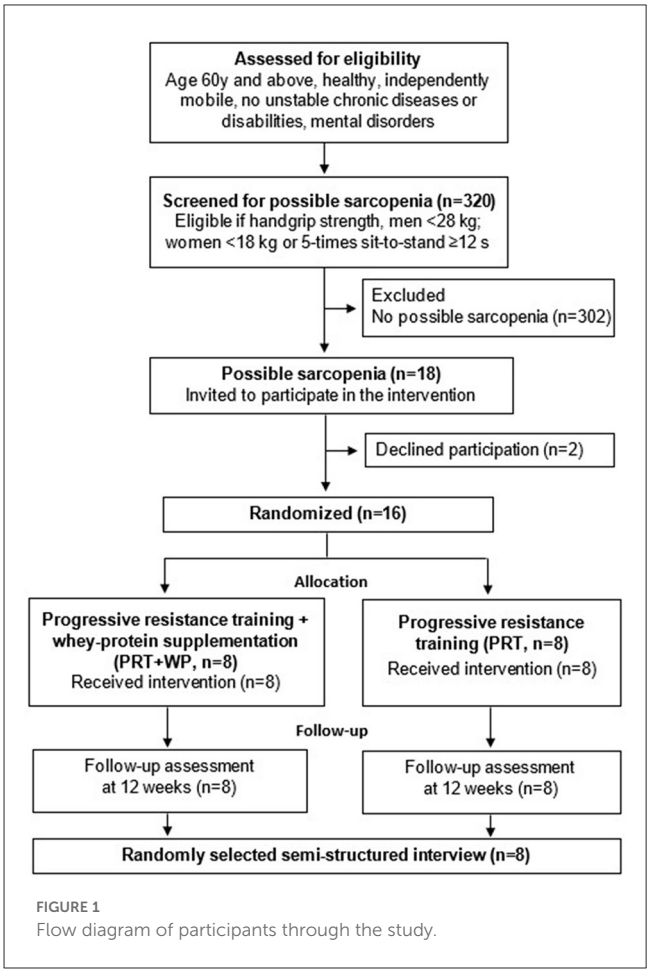

FIGURE 1
Flow diagram of participants through the study.

TABLE 1 Baseline characteristics (mean ± SD) in the progressive resistance training (PRT) plus whey protein supplementation (PRT+WP) and PRT only groups.

Variables	PRT+WP	PRT
n	8	8
Age (years)	66.6 ± 4.0	65.5 ± 1.9
Height (cm)	154.8 ± 5.8	151.6 ± 6.2
Weight (kg)	62.4 ± 10.0	55.9 ± 8.5
Body mass index (kg/m^2)	26.4 ± 4.6	24.5 ± 4.3
Systolic blood pressure (mmHg)	126.0 ± 18.3	134.3 ± 17.8
Diastolic blood pressure (mmHg)	73.5 ± 11.5	71.9 ± 6.7
Resting heart rate (bpm)	78.6 ± 7.4	71.1 ± 11.1

3.2. Prevalence of sarcopenia

At baseline, there were 14 women (88%) with possible sarcopenia (PRT+WP, $n = 8$; PRT, $n = 6$), one (PRT) classified

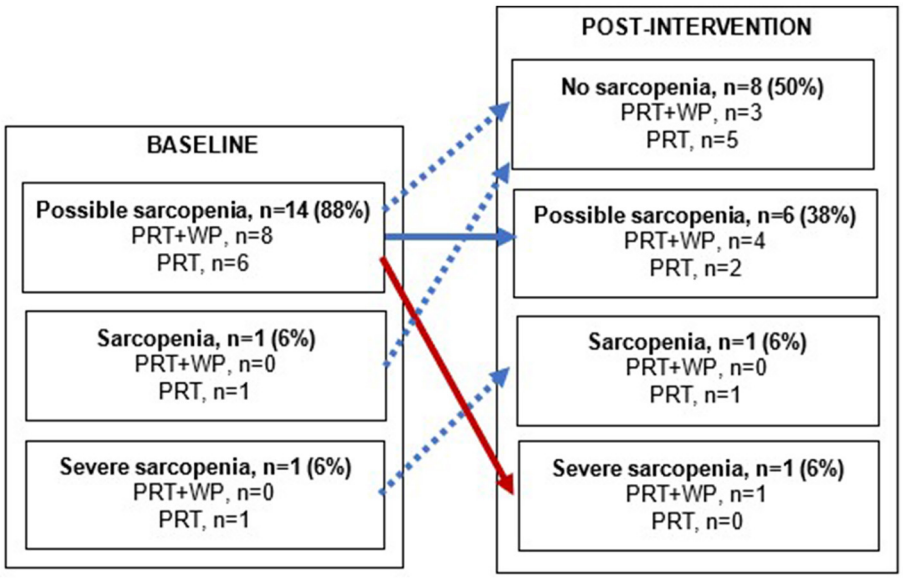

FIGURE 2
Changes in sarcopenia status from baseline to post-intervention within the progressive resistance training (PRT) plus whey protein supplementation (PRT+WP) and PRT-only groups. A solid blue arrow depicts no change in sarcopenia status, blue dashed arrows depict an improvement in sarcopenia status, and a red arrow depicts a decline in sarcopenia status.

as sarcopenic, and one with severe sarcopenia (PRT). As shown in Figure 2, at the end of the intervention, six (38%) women classified as having possible sarcopenia at baseline (PRT+WP, $n = 4$; PRT, $n = 2$) retained the same sarcopenia status, while nine (56%) women (PRT+WP, $n = 3$; PRT, $n = 6$) showed improvements in their sarcopenia status; one woman with possible sarcopenia progressed to severe sarcopenia.

3.3. Muscle strength, mass, and physical performance

There were significant mean changes for PRT+WP in handgrip strength ($p = 0.004$), stair ascent time ($p = 0.03$), and MNA scores ($p = 0.009$), while PRT also had significant mean changes for handgrip strength ($p = 0.008$), stair ascent time ($p = 0.001$), MNA scores ($p = 0.003$), and 5-STS time ($p = 0.03$) (Table 2). However, there were no significant between group net differences for the mean changes between PRT+WP and PRT for any of the sarcopenia components or physical outcomes (Table 2).

3.4. Nutritional status

The PRT+WP group significantly improved their MNA scores ($p = 0.009$) as did the PRT ($p = 0.003$). Although the PRT+WP group's improvement was nearly double the gains compared to PRT, there were no significant differences between the two groups (Table 2).

3.5. Perceptions on PRT and WP

Eight women (5 PRT+WP, 3 PRT) aged 66.6 ± 4.0 years (ranging from 61 to 72) from the completed intervention group took part in the semi-structured interviews. Thematic analysis was used to identify benefits and challenges as perceived by the participants. In total, there were four main themes with sub-themes identified from the data (Figure 3). The data presented are representative of the coding used and the overall findings of the research.

3.5.1. Theme 1: Perceived benefits

Perceived benefits were the most common aspect discussed by the participants, focusing on both the exercise and the high-protein whey-based nutritional supplement. Before starting the intervention, participants were informed about their sarcopenic condition. Perceived benefits were the key driver for them to participate in the study as they were looking for ways to manage and address their condition. Thus, there was no hesitation to participate.

3.5.1.1. Better physical functioning

The benefits derived from the progressive resistance exercise were mainly regarded as a positive outcome as they were beginning to feel better throughout the intervention, especially upon completion. All women were physically inactive before participating in the exercise program and welcomed the idea of incorporating regular physical activity into their daily routine as a result of participating in the study. They also noted that exercising was particularly "scary," and most of them were worried about the

TABLE 2 Baseline (pre) and post-intervention (post) measures in the progressive resistance training (PRT) plus whey protein supplementation (PRT+WP, $n = 8$) and PRT only ($n = 8$) groups and the mean within group changes and net between group differences for the change along with the Cohen's d effect sizes.

	PRT+WP		PRT			
	Mean ± SD or Mean change (95% CI)	d	Mean ± SD or Mean change (95% CI)	d	Net difference (95% CI)	d
Mid-arm circumference (cm)						
Pre	29.0 ± 4.1		27.1 ± 4.1		-	
Post	30.2 ± 4.1		26.4 ± 4.2		-	
Δ (Post–Pre)	1.19 (−0.86 to 3.21)	0.07	−0.66 (−1.96 to 0.64)	0.04	1.85 (−0.33 to 4.03)	0.47
Calf circumference (cm)						
Pre	34.4 ± 4.7		32.1 ± 3.3		–	
Post	33.5 ± 3.4		32.2 ± 4.2		-	
Δ (Post–Pre)	−0.96 (−3.85 to 1.92)	0.06	0.18 (−1.18 to 1.53)	0.01	−1.14 (−4.03 to 1.76)	0.22
ASM (kg/m^2)						
Pre	6.7 ± 0.7		6.2 ± 0.8		-	
Post	6.6 ± 0.8		6.3 ± 0.9		-	
Δ (Post–Pre)	−0.15 (−0.30 to 0.01)	0.24	0.14 (−0.12 to 0.40)	0.18	−0.29 (−0.56 to −0.01)	0.58
Handgrip strength (kg)						
Pre	15.8 ± 1.8		15.1 ± 0.4		–	
Post	20.8 ± 2.7		21.3 ± 4.6		-	
Δ (Post–Pre)	5.0 (2.2 to 7.8)*	0.93	6.2 (2.2 to 10.1)*	0.55	−1.1 (−5.5 to 3.3)	0.14
5-times sit-to-stand (s)						
Pre	12.9 ± 2.9		11.4 ± 1.3		-	
Post	11.6 ± 2.9		9.4 ± 1.4		-	
Δ (Post–Pre)	−1.3 (−3.4 to 0.9)	0.14	−2.0 (−3.8 to −0.2)*	1.04	0.70 (−1.78 to 3.37)	0.17
SPPB score						
Pre	9.1 ± 1.6		9.8 ± 1.3		-	
Post	9.3 ± 1.4		10.0 ± 0.5		-	
Δ (Post–Pre)	0.20 (−1.17 to 1.42)	0.05	0.25 (−0.62 to 1.12)	0.25	−0.13 (−1.56 to 1.31)	0.05
Stair ascent time						
Pre	8.4 ± 0.9		8.1 ± 0.8		-	
Post	7.4 ± 1.3		7.4 ± 0.9		-	
Δ (Post–Pre)	−0.97 (−1.80 to −0.10)*	0.77	−0.75 (−1.0 to −0.45)*	0.97	−0.23 (−1.06 to 0.61)	0.16
Stair descent time						
Pre	8.8 ± 1.6		9.1 ± 1.5		-	
Post	7.6 ± 1.1		8.7 ± 2.0		-	
Δ (Post–Pre)	−1.19 (−2.51 to 0.12)	0.57	−0.40 (−1.13 to 0.34)	0.12	−0.79 (−2.16 to 0.57)	0.32
MNA score						
Pre	24.8 ± 3.2		25.6 ± 2.2		-	
Post	28.2 ± 1.6		27.3 ± 1.7		-	
Δ (Post–Pre)	3.4 (1.2 to 5.7)*	0.52	1.8 (0.8 to 2.7)*	0.44	1.60 (−0.56 to 3.93)	0.44
Well-nourished/Risk of malnutrition						
Pre	4/4		6/2		-	
Post	7/1		8/0		-	

Pre and post-intervention values are mean ± SD; mean within group changes and net different mean (95% CI). *d*, Cohen's effect size with correction; ASM, appendicular skeletal muscle mass; SPPB, Short Physical Performance Battery; MNA, Mini Nutrition Assessment. *$p < 0.05$, within-group change.

FIGURE 3
Themes on the benefits and challenges of participating in a 12-week exercise and nutrition supplementation intervention based on the perceptions of sarcopenic older women.

impact—falling or hurting themselves physically. However, once they started training, their self-confidence increased.

> *"Before, I did not exercise. I used to be tired, but after participating in this research, I feel more energetic. I am getting fitter! My daily movement has become smoother too." (Participant 1, 72 years)*

> *"At first, I was reluctant and was unable to do all the exercises. I was scared that I will fall. I feel better physically now!" (Participant 2, 64 years)*

> *"It was scary for me before starting but I went on to do the exercises because it restored my energy, less muscles pain and I learnt how to exercise properly. After exercising, I felt much more comfortable with my body (laughed)". (Participant 3, 68 years)*

> *"I feel my knees are much better now, l can use squatting toilet now. Happy!" (Participant 5, 61 years)*

> *"It was difficult at the beginning but the longer I did, the easier it was. I am excited to do this exercise every day now as I feel better physically." (Participant 8, 72 years)*

3.5.1.2. Benefits of the nutrition supplement

All women enrolled in the study did not consume any protein-based supplements before joining the intervention. Five women that were interviewed were randomized to receive the nutritional supplement. They perceived themselves as lacking awareness and knowledge on nutrition for sarcopenia and healthy aging. This highlights that more needs to be done to advocate the benefits and importance of high protein-based diets or supplements for older adults with sarcopenia. Some participants indicated that they were worried that it might be similar to past experiences of ingesting milk and milk-based products, which had resulted in bloating and other adverse reactions. However, their overall experience with regard to consuming the 100% whey-based supplemental drink was very positive, and they liked the taste and mouthfeel. There were no adverse effects of the supplement based on self-reported data. More importantly, they stated that they did not experience the similar discomforts they had previously related to milk ingestion. They were ready to include the nutrition supplement in their daily diet as they perceived it to be beneficial for their health.

> *"The milk was very delicious, and I was full after consuming it. As a result, I did not snack much and was able to control my rice consumption. Both milk and exercise helped to improve the body. I feel healthier now!" (Participant 3, 68 years)*

> *"I mixed the milk with chocolate powder at first as normally I get nauseated and bloated tummy if I drink milk. I was surprised it was not like other supplements. The taste was good, and I recommend this product. People like me should be advised to drink this every day." (Participant 7, 64 years)*

> *"I don't drink milk much, but I started to take this supplement. It was delicious, and I can continue to drink it as there were no side effects." (Participant 5, 61 years)*

3.5.2. Theme 2: Sustaining behavior changes

The readiness to change behavior was evident in various aspects discussed with the women. They wanted to change their behavior after learning about sarcopenia and experiencing the intervention. In the long term, they want to improve their lifestyle by exercising and enhancing their knowledge and awareness on nutrition, physical activity, and sarcopenia. The impact of knowing that they were sarcopenic may have influenced their perception, but they were keen to change their behavior related to health. A group or community-based approach rather than an individual approach was perceived as more successful in sustaining their behavior change. Women recruited into this study had responsibilities and routines at home that they were used to, but they willingly accommodated the changes as part of the intervention.

3.5.2.1. Community-based program

There was a preference for the training location to be somewhere where people with similar conditions could meet and exercise together, such as a community-based activity center. They were willing to allocate a certain time of the day to focus on exercising but preferred to attend a specific location to participate in a group activity.

> *"The exercise sessions must be offered to others with the condition… sarcopenia. I felt more energetic and will be useful for others." (Participant 4, 66 years)*

> *"There should be similar exercise sessions for older women in a hall or community center. I am happy to join and will ask my friends to join. We will enjoy exercising together." (Participant 1, 72 years)*

> *"The exercise sessions should be offered beyond the research in groups. I would like to meet others and exercise together!" (Participant 6, 66 years)*

3.5.2.2. Guidance and awareness

Women felt that there was a lack of information on exercise and nutrition for sarcopenia and older adults' health in Malaysia. They learned about nutrition as part of their involvement in the research but would like to have more information. Once they were diagnosed with sarcopenia, they valued greatly any information, especially on nutrition, to ensure their condition did not deteriorate. Dissemination of evidence-based information and guidelines through effective platforms and channels is needed, especially for this age group in Malaysia.

> *"I did not know much about both exercising and nutrition before joining the research." (Participant 1, 72 years)*

> *"I would like to know about exercising, proper food and nutrition for my condition and age as there is not much info out there." (Participant 7, 64 years)*

> *"I would like some guidance on both nutrition and exercising, especially for my condition. I want to feel better, and sure I will take care better if I have the information." (Participant 8, 72 years)*

> *"To be healthier long-term and beyond the research, more information will be valuable and much appreciated." (Participant 2, 64 years)*

3.5.3. Theme 3: Challenges in participating

Study participants highlighted several challenges in the prescribed exercises but perceived that the benefits outweighed these challenges at the end of the intervention. Exercise itself was perceived as a barrier before the intervention. However, the challenges/barriers became their motivation to be more physically active.

3.5.3.1. Types of activities

Certain exercises were challenging to some of the women: chest press, seated leg extensions, and squatting exercises were mentioned. However, self-determination was observed as there were many situations where the exercises were difficult, but all the participants continued and completed most of their prescribed program successfully. At times, they reduced the exercise repetitions but did not stop the exercises. In terms of perceived benefits, how they felt both physically and emotionally upon completing the activities was the main reason for their strong commitments. They felt fitter, were able to move more, felt stronger, and were more confident in doing their daily activities.

> *"Some exercises, such as the chest strengthening, was difficult at the start, but I slowed down at first and later continued as I was able to do more, felt less pain." (Participant 5, 61 years)*

> *"It was difficult to do the squatting exercise. I stopped when it was painful, but by end of the study, I was able to do it! Felt really happy!" (Participant 6, 66 years)*

> *"The chest strengthening was not easy for me, but others were relatively easy. I took more breaks in between but made sure I completed the exercises." (Participant 4, 66 years)*

> *"I felt my muscles were stronger after exercising, and I was able to do my activities like walking and household work better." (Participant 1, 72 years)*

3.5.3.2. Lifestyle and commitments

Lack of time or their current lifestyle was a concern before they started, but they were willing to change their lifestyle to accommodate the activities prescribed during the intervention. This was attributed to their fewer responsibilities due to their age and role at home (retired, not much work to do at home, and no childcare tasks). Therefore, they had a high level of commitment and included the exercises in their usual routine. Participants understood that behavior change was one of the key elements to ensure the sustainability of their actions, especially in the long term. Focusing on activities that emphasize healthy aging was welcomed and appreciated. However, it was not clear and remains to be investigated if the participants continued to exercise beyond the intervention.

> *"I allocated a specific time in the morning and evening, approximately 30–40 min. I was able to commit as I don't have many responsibilities and work around the house." (Participant 7, 64 years)*

> *"I usually set a time after the Asar prayers for about 30 min. The timing worked as I exercised after my daily prayer time. I had a routine that worked for me". (Participant 6, 66 years)*

3.5.4. Theme 4: Wellbeing

All participants wanted to continue with the intervention (exercise and consume the supplement). Improved quality of life and positive emotions were often described as favorable outcomes of the intervention.

3.5.4.1. Emotional wellbeing

Negative emotions such as nervousness, anxiety, lack of confidence, and worry were common thoughts experienced by the women before the intervention. Perceived risk and discomfort were also sources of negative emotions prior to starting the intervention. However, with supervision and guidance from the research team, they were more confident and were able to adhere to the intervention. The perceived benefits were the main motivation

as mentioned in other themes. Participants indicated that they were happier, cheerful, and satisfied, and had a more positive outlook on health, especially by the end of the intervention. Their feelings were attributed to the overall intervention and were not specific to either physical activities or supplementation. Positive emotions experienced also included improvements in quality of life and mental health. Emotions are not easily recognized or widely discussed, but they were evident in this study.

> *"I was doubtful of myself… whether I can do the exercises, but now I am very happy and confident (laughed)!" (Participant 2, 64 years)*

> *"Feeling happier and cheerful every day. I feel grateful for being a part of this research." (Participant 3, 68 years)*

> *"Before I started, I was quite nervous, mainly because I feared getting hurt. However, I was given adequate guidance on how to carry out the exercise. I am glad I took part, feeling more energetic every day now and feel blessed." (Participant 7, 64 years)*

> *"I am more positive now, very happy… maybe because I am more active (laughed)." (Participant 8, 72 years)*

3.5.4.2. Improved quality of life

The overall perception of the intervention was positive as they expected and experienced an improved quality of life. They were more active physically, experienced less pain, and generally felt more positive about their health. All women commented on the improved quality of life due to the intervention. Their perception of quality of life was subjective but likely attributed to being physically active and learning new skills (exercising).

> *"These types of activities should be provided to others too! It has improved how I work!" (Participant 1, 72 years)*

> *"I have a better outlook of my life. I can move around more without pain. I am living better." (Participant 3, 68 years)*

> *"I feel the study is very good. It had a positive impact on my life, such program must continue!" (Participant 4, 66 years)*

4. Discussion

In this study of older sarcopenic Malay women, resistance training (with and without whey-protein-based nutritional supplementation) was associated with many positive perceptive and physical benefits. First, the home-based, high-intensity resistance training program was shown to be safe and effective to optimize muscle health (and even reversing sarcopenia) in older sarcopenic adults. Second, the following four main themes related to commencing and participating in a multi-modal exercise and nutrition intervention emerged: (1) perceived benefits, (2) sustaining behavior changes, (3) challenges in participating, and (4) improved wellbeing that supports the continuation of the exercise and nutrition supplementation. Sub-themes such as better physical functioning and perceived benefits of the nutritional supplementation indicated the positive impact of the intervention, while sub-themes such as preference for community-based programs and being guided and given awareness highlighted their intention to continue with such activities. Challenging activities and how they overcome them were also shared, which are important to inform future implementation. Third, through the interviews, participants' emotional experiences started with fearfulness and doubt about their ability to conduct PRT and consume the WP supplement, but having no adverse effects from both components, the emotions were replaced by a strong sense of achievement following the completion of the intervention.

Previous meta-analyses of randomized controlled trials have shown regular exercise, particularly PRT, or exercise plus nutrition approaches, are effective for improving muscle mass, strength, and performance in older adults with sarcopenia (13, 14). Our intervention was effective in improving muscle strength in both groups, but there were no significant changes in muscle mass. Participants reported completing most of their exercise sessions as we had the COVID-19 pandemic movement control order in Malaysia occur early in the intervention and had to revert to letting participants exercise at home. Participants mainly came to our campus to revise their exercise prescriptions based on their progress and to obtain WP supplementation thereafter. Since the number of enrolled and completed participants in this study was small ($n = 16$), the gains in muscle strength and changes in sarcopenia criterion may be considered case-study findings. Significant muscle strength changes were seen for handgrip values in both groups, and this may be attributed to the action of holding the resistance bands in a grip-like manner. Stair ascent time improved significantly by 0.8–1.0 s in both groups and 5-STS time by 1.3–2.0 s, which was significant for the PRT group. Thus, other than knowing which type of exercise works for sarcopenic older adults, participants' insights on how it may have worked for them are key to implementation.

Lack of knowledge on the health benefits of nutrition and exercise in older adults was a barrier to participation, as identified in a sarcopenia-related study (29). This was also evident in this study where participants identified the intervention as a preventive measure on top of managing sarcopenia. Participants wanted more guidelines and information on nutrition and recommended exercises to prevent or treat sarcopenia to be disseminated to them and their community. Information dissemination and awareness of sarcopenia are vital, and this includes having the medical fraternity be able to identify sarcopenia in their older patients (30). However, it remains to be explored how such information can be shared effectively and more widely. Future research could consider using digital tools and platforms and educating medical and health professionals about the importance of screening and diagnosing sarcopenia and how it can be treated. Another possible strategy is to apply implementation research or knowledge-translation study designs to assess the delivery of effective, scientific findings to the targeted community (31).

Reasons for non-participation in physical activity and exercise commonly include sociodemographic factors, including ethnicity, education level, and employment status (17). However, women

in our study did not refer to their sociodemographic background as the main reason for not being physically active prior to participating in the research. Rather, it was the knowledge of their muscle condition or ability, i.e., sarcopenia, that largely determined their participation. Correspondingly, they focused on the perceived benefits that were achievable from participating in the intervention. Other concerns regarding participating in the exercise include lack of confidence and fear of falling or getting hurt, which are very real and are associated with less independent mobility and increased frailty in older adults (32, 33) and this was echoed by our participants. However, participants in this study were motivated to complete the intervention because they experienced positive change and felt better physically. Quantitatively, the change in stair ascent time was statistically significant and is not a criterion in sarcopenia but may be reflective of how these women feel after the intervention period as they could climb up the stairs at a faster pace. These self-reported improvements or patient-reported outcomes described the context of meaningful change that may be more relevant to people with sarcopenia (34). Overall reasons for participating and continuing with the intervention echoed the Health Belief Model along with other health behavior and social cognitive theories (35), where health motivation and perceived benefits (professional-guided PRT and suitable nutrition supplement) superseded their perceived barriers (fears and doubts) in managing sarcopenia. Through this study, we could see that we have unintentionally addressed components within behavioral change models to witness the change in participants' physical activity levels.

Both physical activity and nutrition are crucial in maintaining skeletal muscle mass and function in older adults (13, 14, 36). There is increasing research recognizing the critical role of increased dietary protein or protein supplements in combination with physical activity to prevent sarcopenia (37–40). Although milk consumption among older adults in Malaysia is considered uncommon (41), the high-protein whey-based supplement drink used in this study was well accepted due to its taste and limited side effects. Although there were no differences between PRT+WP and PRT groups, which is likely due to the small sample size, we also did not account for habitual dietary intake in this study and could not establish if either group had sufficient or inadequate nutrient intake. However, the MNA scores improved in the PRT+WP group, which also had more participants within the at-risk of malnutrition category at baseline. However, due to the small sample numbers, these findings are case-study-level evidence at best. The main messages from the personal perceptions of the participants were that: (1) the compliance with the nutritional supplement in this study was high (90%) and (2) the women enjoyed consuming the drink, which suggests that such an approach can be used to increase protein intake in older Malay women. Older adults can be encouraged to try whey-based products as an alternative to whole-milk products to minimize unpleasant experiences with normal milk intake and thus add good-quality proteins and nutrients to their diet.

Participants in this research who completed their exercise program at home indicated that they would prefer to attend a group-based, community program rather than exercising entirely by themselves at home, but they did not discuss any potential barriers or support needed to attend such programs. Barriers to attending a community-based program should be a focus area in future research as factors such as affordability and costs related to fees for exercise professionals to conduct programs were barriers to attending exercise sessions in several previous interventions (42, 43). However, research elucidating the types of exercise interventions for older adults remains inconclusive as the setting (clinical vs. community vs. home-based), qualified professional supervision, multimodal program components, or even group vs. individual-based program components influence long-term adherence, sustainability, and preferences across different populations (44). Researchers have highlighted the social aspect of a group program as a powerful driver for adherence and future continuation of being physically active (44), which is consistent with some of the findings from our study.

Other than health benefits, engagement in physical activity in older adults stemmed from feelings of being included; social engagement and being part of something that is accessible were major components as addressed by older adults in the literature (45). Our participants highlighted their preference to continue the PRT and WP programs in a community-based setting that evokes a similar sense of inclusiveness and social bonding through the activity. More specifically, participants were willing to allocate specific time to exercise and were motivated to continue with the exercises beyond the intervention. In this study, words such as "happy," "blessed," and "excited" were used to describe how they felt and were associated with how they perceived improvements to their physical health. Positive emotions can be enhanced and sustained by focusing on community-based physical activities as previous research has shown that community settings result in the enjoyment of physical exercises and positive emotions from the consequences of social networking (38). Participants' eagerness and motivation to engage were also evident in our study, which is crucial to ensure sustained engagement (46).

Sustained participation is related to having the opportunity to form social bonds and working closely with peers that will encourage and motivate one another (29), which was also mentioned by our participants. One study reported that some ethnic groups often focus on cultural sensitivity; for example, some Muslim women would not exercise in groups with men due to religious practices requiring gender segregation (42). However, this was not highlighted as an issue in our study, despite the fact that all were Muslim women. There is an interest in gender-specific differences in the development of sarcopenia (29), and although participants were very keen to change their behavior to be healthier with the strong advocation for structured exercise sessions, our study is limited to views from women only as no men participated in this study.

Encouraged by the findings from this pilot trial of the PRT and WP interventions that were both quantitatively and qualitatively analyzed, a more comprehensive implementation type of research is required. Note that our study results were limited to views and physiological responses from a small all-women cohort, and the results should be interpreted with caution. Furthermore, during the study, we had challenges with access and logistics due to the COVID-19 movement control orders in Malaysia which may have reduced our efficiency to effectively monitor and determine compliance with all the PRT sessions and supplementation intake. However, physical changes were evident, and participants would

meet to assess progression, change exercise bands, and receive the next dose of the whey protein supplementation. Although we monitored participants via weekly phone calls, there may be self-report biases as one individual deteriorated to severe sarcopenia despite reporting completing the prescribed exercises and supplementation. Furthermore, our study only included one ethnicity (Malays) in Malaysia, and thus, future research should include multiple populations and settings to capture a more inclusive perception. Finally, the study design was geographically restricted to Malaysia's East Coast, but the methods can be replicated elsewhere to include wider sociodemographic factors and ultimately capture the views of the culturally diverse population in Malaysia.

5. Conclusion

In this study of older Malay women with sarcopenia, the overall physical outcomes were encouraging, and the experiences reflected upon by the women completing the 12 weeks of exercise and nutrition intervention were positive, with valuable recommendations to inform future interventions and in designing prevention activities within a community setting, especially in Malaysia. While the women were initially skeptical and fearful of participating in a multi-faceted exercise and nutrition program, they reported a positive experience following the intervention due to the perceived physical and mental health benefits. The changes they made to their lifestyle for 12 weeks as part of the intervention were something that they were willing to sustain in their daily lives with adequate support and guidance. However, to facilitate future implementation and participation, women indicated they want more multi-model exercises, nutrition guidance, and programs that are incorporated into community-based settings. Collectively, the findings from this study indicated that engagement in physical activities and subsequently preventing sarcopenia among older adults can be successful with the right support, facilities, and guidance that can be investigated within an implementation research design.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by Human Research Ethics of Universiti Sains Malaysia. The patients/participants provided their written informed consent to participate in this study.

Author contributions

VT conceptualized, conducted formal analysis and investigation of the study, drafted, and reviewed the manuscript. RV performed investigation and data analyses, prepared original draft of the manuscript, reviewed, and edited the manuscript. RD provided research resources, reviewed, and edited the manuscript. All authors read and approved the final manuscript.

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Conflict of interest

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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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2023.1176523/full#supplementary-material

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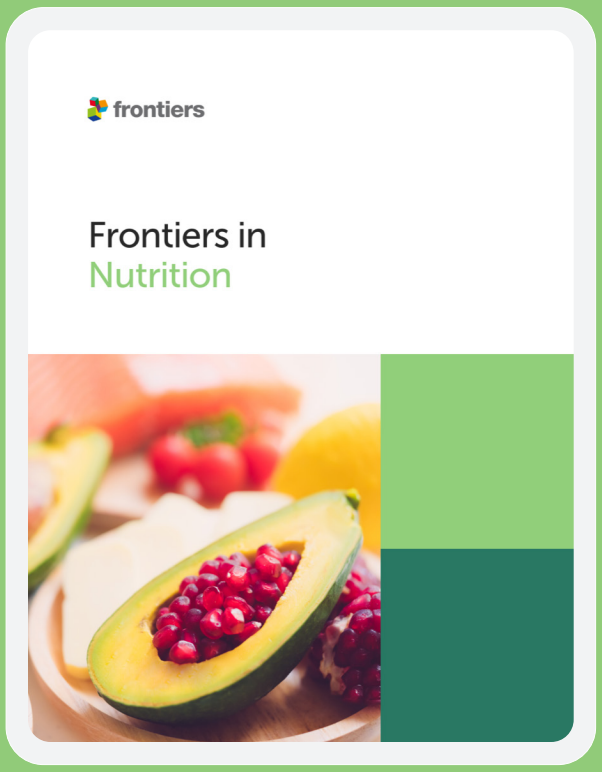

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