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Space countermeasures and medicine - implementation into earth medicine and rehabilitation

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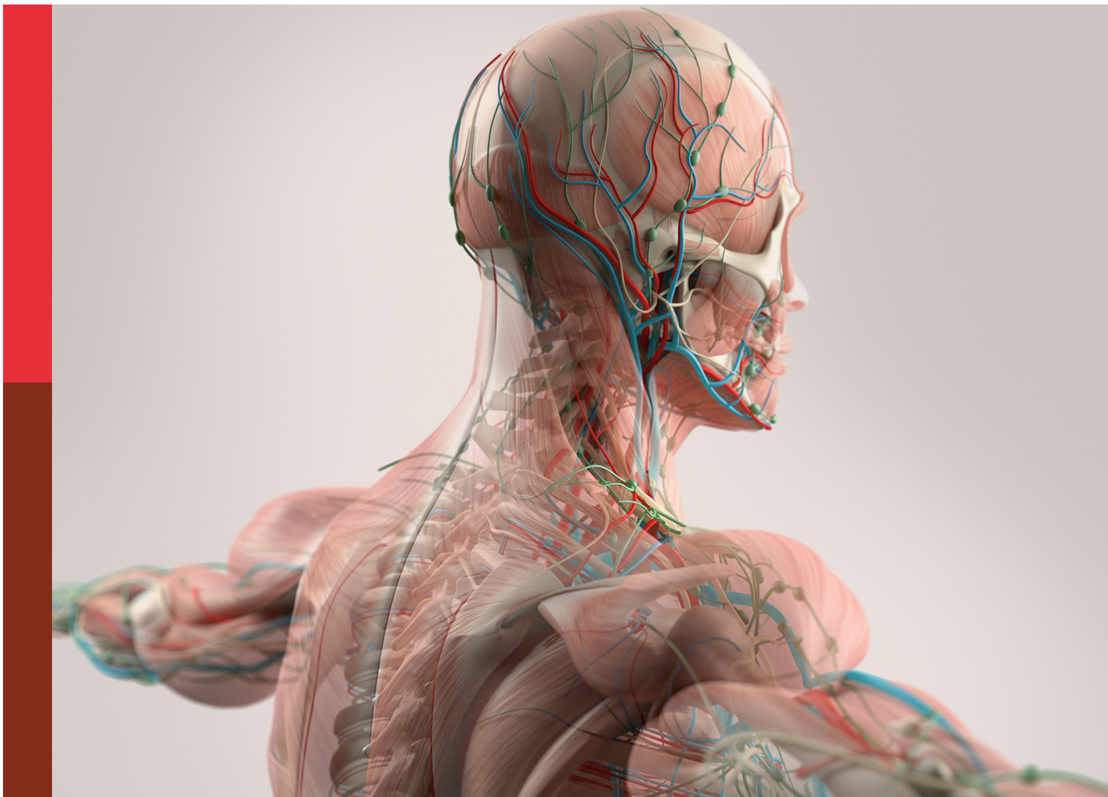

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Space countermeasures and medicine - implementation into earth medicine and rehabilitation

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OPINION
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Possibilities of Proteomics Profiling in Predicting Dysfunction of the Cardiovascular System

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INTRODUCTION

In our opinion, cardiovascular proteomics is the most perspective field of proteomic research in space and Earth medicine. Proteomics, being still a relatively new discipline of fundamental research, is becoming not only a methodology for screening potential biomarkers in various cells, tissues, and biological fluids but also a method for determining the targets of therapeutic agents (Huang et al., 2017). Widely, understanding how multiple protein species interact to carry out regulation is an important objective of cardiovascular research. Besides, the power of proteomics to simultaneously provide information on the panoply of expressed proteins has made it uniquely suitable for resolving complex signaling conundrums and revealing cardiovascular diseases (CVD). (Lam et al., 2016). When using proteomic analysis in the cardiovascular system (CVS), two main strategies are relevant (Mokou et al., 2017): obtaining information about molecular physiological mechanisms and identification of biomarkers that reflect the dynamics of adaptation processes.

The variety of CVS adaptive responses is ensured by the high variability of the metabolic circuit of regulation of this system since numerous molecular mechanisms are involved in this process and, first of all, in the regulation of heart rate. From a physiological point of view, heart rate variability (HRV) is an integral characteristic that reflects not only the adaptive response of the CVS (Thayer and Lane, 2007; Thayer et al., 2010; Ernst, 2017) but also of more complex neural networks (Thayer et al., 2012).

However, the relationships between HRV and metabolic parameters under conditions of space flight (SF) conditions and clinical studies are poorly understood.

PROTEOMIC PROFILING IN SPACE FLIGHT, GROUND MODEL EXPERIMENTS, AND CLINICAL STUDIES

In our studies, we tried to describe some proteomic markers of the human cardiovascular system in SF and in-ground model experiments.

We have analyzed (Pastushkova et al., 2019) the characteristics of the HRV which can be reflected in the urine proteome in cosmonauts with different initial types of CVS regulation. In this report, we revealed that the concentration of three proteins: alpha-1 of collagen subunit type VI (COL6A1), mucin-1 (MUC1), cadherin-13 (CDH13), hemisentin-1 (HMCN1), semenogelin-2 (SEMG2), SH3 domain-binding protein (SH3BGRL3), transthyretin (TTR) and serine proteases inhibitors (IPSP) significantly differ between groups of cosmonauts with predominance parasympathetic or sympathetic tone. In our point of view, this fact characterized different strategies of adaptation in cosmonauts with different types of the predominance of autonomic tone.

In our next report (Pastushkova et al., 2020), these differences were confirmed by the fact that some biochemical parameters, were unidirectionally changed with three proteins on the 1-st and 7-the days after SF. These sets were totally different in classified groups. In group of cosmonauts with a predominance of the sympathetic tone were changed unidirectionally COL6A1 with potassium, ferrum, alpha-1. MUC1 with amylase, urea, inorganic phosphate, glucose, alkaline phosphatase, ionized calcium. CDH13 with uric acid, ferrum, alpha-1, potassium. In group of cosmonauts with a predominance of the parasympathetic tone: direct bilirubin, potassium, total calcium with COL6A1. Direct bilirubin, potassium with MUC1 and total ferrum biding capacity, transferrin, glucose gamma, globulin transferase with CDH13.

We hypothesized that the concentration of these proteins and their different relationship with some biochemical parameters reflect, as we called this process, "adaptation price" which depends on the type of autonomic regulation.

In some cases, studies in ground model experiments may be more productive than studies in the SF. Under conditions of 120-days isolation, we studied the participation of collagens, which are proteins of the extracellular matrix (ECM) and participate in the modulation of the biomechanical characteristics of the CVS. Our next report (Rusanov et al., 2022) presents these results. We hypothesized that collagens may be a biomarker of modulating influences of regulatory mechanisms of the circulatory system and also be markers of the aging process.

In-ground model experiments with "dry immersion" we identified a set of proteins with consisted of cell adhesion molecule 4 (CADM4), immunoglobulin heavy alpha-1 (IGHA1), serotransferrin (TRFE), tyrosine-protein kinase receptor (UFOAXL), galectin-3-binding protein (Gal-3BP) and matrix remodeling-associated protein (8MXRA8) and reflects, in our opinion, autonomic regulation of the cardiovascular system (Rusanov et al., 2020a). Moreover, correspondence of the results of an assessment of the direction, as well as the response time of various circuits of blood circulation regulation, which reflect their reactivity as an indicator of the adaptive capabilities of the body, has been demonstrated. It is shown that the results of the assessment of directivity, as well as the response time of various regulatory circuits in CVS, differed in the time range (Rusanov et al., 2020b).

The use of proteomic approaches, tested in space medicine, in clinical practice in patients with CVD would make it possible to expand the understanding of the development of the pathological process, which can serve as the basis for developing an individual treatment strategy, since a number of studies have shown high variability between people for various molecular indications, and individual initial variability was low (Tebani et al., 2020).

In an earlier publication (Pastushkova et al., 2017), we have profiled the urine proteome of 18 healthy subjects and compared it with the urine proteome profile of 18 patients with ischemic heart disease accompanied by hypertension. Comparison of urine proteome of healthy people and patients with postinfarction cardiosclerosis revealed proteins specific for patients with cardiovascular disease. Thus, proteins vitronectin (VTN), syndecan-4 (SDC4), a histidine rich glycoprotein (HRG), endothelial protein C receptor (EPCR), colony stimulating factor (CSFs), cathepsin D and sekretogranin-1(CTSD) may be considered (GHGB) as potential markers for CVD.

CONCLUSION

Further research in the direction of cardiovascular proteomics should concern the clinical and experimental verification of the hypotheses. For public health, research in cardiovascular proteomics would help identify possible candidate proteins for more effective treatment and diagnosis of CVD. Furthermore, in our opinion, the identification and profiling of personalized metabolic markers is the way to personalized medicine.

Undoubtedly, proteomic research in space medicine has one serious limitation. The concept of a biomarker was proposed in 2001 by the US National Institutes of Health and includes a characteristic that can be objectively measured and that can serve as an indicator of physiological and pathological biological processes (Hoefner, 2001). Biomarker needs to be verified in large cohorts. The cohort of cosmonauts and even volunteers participating in model ground experiments is too small for this. However, we can try to identify proteins associated with the cardiovascular system and describe the signaling pathways of these proteins for further study as potential markers of physiological or pathological processes in CVS.

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All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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OPINION
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Using the Possibilities of Russian Space Medicine for Terrestrial Healthcare

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INTRODUCTION

During the operation of orbital stations, the methods and means of medical support for cosmonauts and astronauts, monitoring their health status are being constantly improved, knowledge about the capabilities of the person himself, about the methods for managing the processes of human body adaptation to changing and often harsh environmental conditions, is increasing. It is well known that the consequences of the impact of various spaceflight factors are also encountered to some extent in terrestrial life—hypodynamia, hypokinesia, increased background radiation, deafferentation, isolation, etc. That is why the current level of development of biomedical research makes it possible to use the results obtained to keep the health of people not only in space, but also on Earth (Space Physiology . . . , 2016; Grigoriev, 2007). Dietrich et al. showed in their review that space technology has an impact on many domains of activity on earth, including in the field of global health. Various health research and technologies developed for inhabited space flights have been adapted for terrestrial use (Dietrich et al., 2018).

The Institute of Biomedical Problems of the Russian Academy of Sciences (IBMP RAS)—is the leading institution in Russia in the field of space biology and medicine. It is responsible for medical and sanitary and hygienic support for the crews, as well as the creation of scientific equipment for solving the problems of medical support and the implementation of the Russian national program of biomedical research and experiments on the Russian segment of the International Space Station (ISS). In addition, the IBMP RAS conducts interdisciplinary fundamental and pilot research in the field of medical sciences, radiobiology, engineering science, biotechnology, etc.

The Institute has carried out significant scientific and applied research, obtained unique results, and developed modern equipment (Belakovskiy and Samarin, 2002, 2011; Space Medicine . . . , 2014). It is obvious that the main area of the research results implementation is the improvement of medical support for the health and performance of cosmonauts in-flight and after returning to Earth, but nevertheless, a significant part of them has practical importance for implementation in healthcare(Orlov et al., 2014; Orlov et al., 2021).

DIRECTIONS FOR USING THE ACHIEVEMENTS OF SPACE MEDICINE

In terms of using the possibilities of space medicine and its achievements to ensure the health and treatment of people, there are several areas in which a targeted search is underway:

1) Extension and deepening of knowledge about human health.

2) The use of equipment and research methods used on board space-based platforms in healthcare practice, i.e. adaptation of means, methods, equipment and devices created to solve the problems of space medicine to the tasks of earth medicine.
3) Application of space technologies in medical practice.

The tasks of space medicine include managing the functions of the human body in extreme environmental conditions to ensure a high level of its performance and the obligatory maintenance of an optimal state of health. This research area has generated qualitative shifts in the approaches and methodology of modern medicine. The main idea is that practically for the first time a healthy person became the object of study for a doctor. Multilateral systematic examinations, the most detailed study of all life processes occurring in a healthy human body, have enriched medicine with knowledge of normal reactions to various environmental influences. Maximum consideration of the body's reserves, an individual approach, the use of the most modern methods of medical science for remote monitoring and predicting the state of health, the search for the border between adaptive and pathological changes under the influence of extreme environmental factors, and the prevention of the consequences of these factors distinguish precisely the space branch of medicine. All this allows to better know the normal human physiology. It can be argued that the knowledge of "terrestrial" physicians about the mechanisms of a person's spatial orientation, the vestibular apparatus, its structure and function, information about biomechanics, metabolism, cardiovascular and nervous systems was replenished due to the cosmonautics.

The most famous examples of such "enrichment" of knowledge are the problems of hypokinesia and "motion sickness" (kinetosis). Hypokinesia was one of the effective ground models for simulating the physiological effects of weightlessness. By now, many studies have been carried out, including those with the length of stay of a healthy person on bed rest up to one year. In these studies, not only many data were obtained on the state and functions of all systems of the human body under these conditions, but also various methods and means of correcting the ongoing changes, as well as complex rehabilitative measures, were tested. All this, together with the available clinical material, provide a complete picture of the pros and cons of keeping patients in bed for a long time. Kinetoses, which are a very acute problem of cosmonautics, have prevented many people from traveling freely using air, sea, and even road transport. Pharmacological agents developed for cosmonauts for the prevention and reduction of manifestations of the space form of kinetosis show their effectiveness in other forms of motion sickness. A complete solution of this problem will be a serious contribution not only to cosmonautics, but also to practical public health. (Grigoriev et al., 2001a; Grigoriev et al., 2001b; Grigoriev et al., 2002; Grigoriev et al., 2003; Grigoriev et al., 2004; Grigoriev et al., 2005a; Grigoriev et al., 2005b; Vinogradova et al., 2005; Grigoriev et al., 2008; Grigoriev et al., 2009; Kozlovskaya et al., 2009; Grigoriev et al., 2012; Shenkman et al., 2014; Kozlovskaya et al., 2019).

The results of studying the functions and performance of humans in space under extreme environmental conditions have deepened our knowledge of the individual characteristics of healthy people adaptation, of determining the criteria for normal and pathological reactions of humans in these conditions, as well as the first symptoms of pre-illness. The accumulated experience is used to study the contingents of people whose professional activities take place in extreme conditions. A comprehensive assessment of indicators characterizing the functional state of the body, in particular its adaptive capabilities, makes it possible to identify the body's response to the environmental situation not after 4–5 years, when diseases occur under the influence of adverse environmental conditions, but in the first months, as soon as the primary reaction of the organism develops as a general adaptation syndrome. (Baevskiy, 1979; Baevskiy et al., 2002; Baevskiy et al., 2008; Orlov et al., 2013; Baevskiy et al., 2014).

Space medicine has developed and implemented in healthcare new criteria and standards for human tolerance to functional loads, such as dosed physical activity on a veloergometer, passive orthostatic and antiorthostatic tests, a test with negative pressure on the lower half of the body.

Finally, valuable experience was gained in spaceflights in solving social and psychological issues of ensuring the professional activities of small groups of operators in extreme conditions of isolation and stress, optimizing their interaction, work regime, and organizing psychological support measures. All this is directly related to the problems of "terrestrial" sociology.

Biological and medical devices designed for human spaceflights have some advantages over existing "ground" devices: portability, low weight, easy use, resistance to overload, shock, vibration, and temperature changes. These devices are competitive in the class of equipment for the organization of a medical service that provides emergency assistance directly in sites of natural disasters, catastrophes, for medical examination of the population in hard-to-reach areas, for underwater, aviation and marine medicine, as well as for the examination of athletes (Grigoriev et al., 2003; Baevskiy et al., 2008; Netreba et al., 2009a; Netreba et al., 2009b).

An important direction in the application of the unique experience of space medicine for the benefit of humanity is the widespread use of telemedicine (Telematics Systems . . . 1992; Krupinski et al., 2002; Wilhite et al., 2022). Many years ago telemetry facilities were successfully developed and used in space medicine. The main purpose of these systems was medical control in order to recognize deviations dangerous to the health of cosmonauts. Even now it is difficult to imagine a more remote access of a doctor to a "patient", although quite healthy. Of course, modern computer technologies have greatly modified the possibilities of telemedicine access on board the space station, but they are now being used more successfully in real-time medical teleconferencing and delayed consultations.

In almost all countries, there is a real need for reliable communication systems capable of transmitting medical information. Russia is one of the countries that, due to geographical features and a number of other factors, needs most to introduce such systems and technologies into the practice of medicine and healthcare (Kamaev et al., 2001; Clinical telemedicine . . . , 2001). Consulting patients from remote regions by specialized medical centers of the country, creating a more effective system of primary, postgraduate and continuing medical education, organizing and conducting coordinated research programs on the most actual problems, timely and targeted medical response to disasters and crises - this is an incomplete list of the main directions for application of telemedicine in clinical practice (Grigoriev et al., 2003; Perevedentsev and Orlov, 2012; Grigoriev and Sarkisyan, 1996).

PLATFORMS FOR TESTING AND USING THE DEVELOPMENTS OF SPACE MEDICINE

The path from an idea to the implementation of a technology in clinical practice can take a long time and require a lot of resources. That is why one is interested in the mechanisms to speed up this process. An effective platform for testing technologies and adapting them for the clinic is ground-based analog studies. They allow such testing to be carried out in close interaction between science and technology, providing prompt feedback for developers, as well as working out organizational mechanisms for interaction between scientific organizations and industrial enterprises. The IBMP RAS has the capacity to conduct a wide range of analog studies (isolation, dry immersion, hypokinesia, short-arm centrifuge, etc.).

That is why it was invited to join the consortium, on the basis of which the World-Class Scientific Center (WCSC)—Pavlov Center "Integrative Physiology for Medicine, High-Tech Healthcare and Stress Resistance Technologies" was created. WCSCs are consortiums created within the framework of the Russian national project "Science" on the basis of an open competitive selection for grants in the form of subsidies from the federal budget. They were created in priority areas of scientific and technological development. The Pavlov Center included the Institute of Physiology named after I.P. Pavlov, Institute of Biochemistry RAS (as Coordinator), IBMP RAS, Institute of Evolutionary Physiology and Biochemistry named after I.M. Sechenov, St. Petersburg State Electrotechnical University "LETI" named after. V.I.Ulyanov (Lenin). Each element of the consortium has its own role. Thus, the Center for the Study and Prevention of the Effects of Long-Term Isolation was established at the IBMP RAS. Its purpose is to study the problems of stress caused by long-term physical and social isolation on the basis of model experiments and develop approaches to their prevention. These studies will allow substantiating the methods of psychological support, as well as suggesting new methods and modes of electromyostimulation and gravitational therapy for clinical practice. Thus, WCSC can serve as a successful example of mechanisms for adapting space technologies for the clinic.

However, further production, market launch and sales of products using registered intellectual property require a significant investment of resources, the availability of specialized competencies, as well as work in the appropriate legal field. Several mechanisms can be used to implement these processes. These include the following:

1) Providing advisory and expert support to small and medium-sized companies existing in the market,
2) Creation of small enterprises and conclusion of licensing agreements for granting non-exclusive licenses for the use of inventions (this is how several license agreements were concluded between the IBMP RAS and Center for Aerospace Medicine LLC, TsAM LLC) or the conclusion of agreements with already existing companies,
3) Creation of small innovative enterprises with the transfer of the right to use the results of intellectual activity (for example, contributions to the authorized capital) (this is how the Innovation Center of Space Medicine LLC was created).

Each of these mechanisms contribute not only to the expansion of IBMP's opportunities for technology transfer, but also allow choosing the best way for such a transfer.

DISCUSSION

The application of methodological and technical developments of space medicine opens up unique opportunities for practical healthcare, changing the approaches to the structure of the medical care organization system. Space physiology and medicine provides rich material for creating methods, devices, technologies and knowledge that have the potential to improve the system of organizing medical care for the population of our country, and develop international cooperation in the field of practical healthcare. At the same time, the existing system for obtaining and applying scientific knowledge was initially not adapted to the large-scale implementation of research results into practice. In the process of its development, several working mechanisms of varying degrees of prevalence and effectiveness were worked out. The current mechanisms for testing and creating new technologies for healthcare based on existing space developments (research within the framework of the WCSC, the creation of SIB, etc.) make it possible to test, modify and find application for scientific developments, in particular, developments in space biology and medicine. The choice of a mechanism is influenced by a whole range of factors - from the prospects of development and the amount of necessary costs for the creation and implementation of a method or technology to the organizational and legal form of a scientific organization and the involvement of researchers and developers in the process of transferring technology into practice. In any case, the mechanisms require constant improvement, development and adaptation to the changing conditions of the economic environment. In order to make innovative work in this area more meaningful, effective and dynamic, it is necessary to form a social order, as well as more actively involve non-state structures that could suggest the most promising directions of the commercialization projects, from their point of view. This is what will allow the introduction of the developed "flight" technologies into clinical practice and biotechnological area to remain a significant component of medicine in the 21st century.

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Application of Space Technologies Aimed at Proprioceptive Correction in Terrestrial Medicine in Russia

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Space technologies greatly contributed not only to space medicine but also to terrestrial medicine, which actively involves these technologies in everyday practice. Based on the existing countermeasures, and due to similarities of sensorimotor alterations provoked by the weightlessness with various neurological disorders, a lot of work has been dedicated to adaptation and introduction of these countermeasures for rehabilitation of patients. Axial loading suit and mechanical stimulation of the soles' support zones are used in mitigation of stroke and traumatic brain injury consequences. They are also applied for rehabilitation of children with cerebral palsy. Complex application of these proprioceptive correction methods in neurorehabilitation programs makes it possible to effectively treat neurological patients with severe motor disturbances and significant brain damage.

Keywords: Proprioceptive correction, support afferentation, neurorehabilitation, Korvit, axial loading suit

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1 INTRODUCTION

Biomedical support for manned space missions is comprised by advanced medical achievements. Not only its development upgraded existing medical technologies, but it also led to invention of unique methods and approaches. The major focus of aerospace physiology and medicine revolved around prevention and reduction of space flight associated negative effects, mostly around the effects of microgravity and hypokinesia. All the biomedical data obtained during space flight and ground-based model experiments led to better understanding of physiological characteristics and capabilities of human body in the conditions of real and simulated microgravity. In addition, space biomedical research enlarged existing knowledge of how the body adapts to the conditions of ambient environment and the underlying mechanisms of this adaptation. This helped to create new ergonomic and reliable equipment and techniques that can be used in extreme conditions and confined environment. The importance of these space developments and their customized versions is also in their integration with terrestrial medicine, as they are applied for diagnosis establishment, treatment and prevention of various diseases and rehabilitation therapy (Legostaev, Markov and Sorokin, 2013; Orlov, Belakovskiy and Kussmaul, 2014; Orlov, Kussmaul and Belakovskiy, 2020).

2 SPACE TECHNOLOGIES IN NEUROREHABILITATION

Even though scientific and technological progress simplified human life in general, it also triggered certain problems, such as deprivation of motor activity, which resulted in increasing numbers of people suffering from various disorders of musculoskeletal system. Nowadays hypokinesia is a vexed problem of all age groups and is considered to be among the main causes of various metabolic disorders (metabolic syndrome, obesity, type II diabetes mellitus), diseases of cardiovascular system (ischemic heart disease, atherosclerosis, hypertension), sarcopenia—an age-related decrease in muscle mass and functional capabilities. Besides, there are people with forcedly limited physical activity, such as patients who need to maintain prolonged bed rest and who are especially susceptible to negative influence of hypokinesia factors (Grigoriev, 2001; Popov, 2018). In the past few years scientists have acquired significant amount of data suggesting that hypokinesia and hypokinesia-related changes in the regulatory and metabolic mechanisms intensify the course of pathological processes. Support withdrawal (weightlessness) and a decrease in volume of support stimuli (hypokinesia) strongly reduce slow-twitch (tonic) motor units' activity. This reduction is always accompanied by muscle atony and absolute muscle strength decline (Kozlovskaya et al., 2007). In fact, the more the percentage of slow-twitch fibers, the higher the decrease of absolute muscle strength. Exposure to microgravity and full elimination of support provokes a condition called hypogravity motor syndrome: short-term exposure results in decreased muscle tone and muscle contractile capabilities (Kozlovskaya, 2018). Along with that, it provokes osteoporosis (demineralization of bone tissue), body ataxia and apraxia, alters body scheme perception and movement biomechanics. It also induces coordination disorders manifested by a sharp decrease in vertical stability, postural synergy system disruption, and changes in the structure of motor acts (Williams et al., 2009).

Due to similarities of sensorimotor alterations provoked by weightlessness and various pathological disorders, a lot of work has been dedicated to adaptation of existing space countermeasures for rehabilitation of patients, suffering from severe motor disturbances, triggered by children cerebral palsy, ischemic stroke, brain injuries and other spinal and cardiovascular pathologies (Kozlovskaya et al., 2006, 2007).

3 AXIAL LOADING SUIT FOR NEUROREHABILITATION

While preparing cosmonauts for long-term space flights, the Institute of Biomedical Problems of the Russian Academy of Sciences and "Zvezda" Research and Production Association developed "Penguin" axial loading suit. The main purpose of this suit was to create axial load and compensate the absence of support and proprioceptive afferentation in microgravity and during hypokinesia, thus mitigating negative effects of weightlessness. Action of the suit is associated with direct influence on proprioceptors of muscles and joints and with simultaneous correction of afferent vestibulo-proprioceptive flow on the central structures of motor control brain areas. Motor afferentation has a profound activating effect on human brain: the flow of proprioceptive stimuli changes the functional properties of neurons, transforms them into polymodal neurons and provides them with increased susceptibility to stimuli of various sensory modalities. The suit has a system of elastic loading elements, which are distributed according to the antagonist muscles' topography. The suit corrects initial posture asymmetries and limits body's excessive degrees of freedom by creating load on musculoskeletal system and increasing resistance for movement performance (**Figure 1**) (Yarmanova et al., 2015).

In the early 90s, "Penguin" was modified into "Adeli" medical suit and then used in a dynamic proprioceptive correction method. This method was first used in 1991 as a complex treatment of cerebral spastic infantile paralysis at the Institute of Pediatrics of the Russian Academy of Sciences (Semenova and Antonova, 1998). This method allowed children with severe motor defects to develop walking skills in a shorter period of time, improve vertical stability, posture, functional state of neuromotor apparatus and intellectual functions (Semenova, 1997; Nemkova SA. et al., 2000; Nemkova S. A. et al, 2000). "Regent" medical suit (a "Penguin" suit modification) was used for rehabilitation of patients with motor disorders caused by stroke and traumatic brain injury. Design of the "Regent" suit is different from the above mentioned models, as its elastic loading elements can be removed from the suit and reattached in diverse ways. Such variability in their distribution allows to create multidirectional load on the body (Galanov et al., 2010; Makarova et al., 2012). "Regent" suit is most frequently used for neurorehabilitation nowadays. The complex influence of this suit is defined by a number of factors:

- Axial load on the musculoskeletal system, which is important for patients exposed to hypokinesia
- High resistance for performing movements
- Gait and posture improvement
- Limitation of joint and ligament hypermobility
- Sole compression to counteract its pathological disposition
- Increase in intensity of proprioceptive input
- Ability to develop personalized treatment plan according to patient's movement disorders, due to simplified suit regulation and adjustment (Galanov et al., 2010).

3.1 Post-Stroke Rehabilitation

Instability and imbalance are serious problems for patients with cerebral stroke. Even in the absence of paresis, patients who had a vertebrobasilar stroke often suffer from pronounced impairments in the function of movement, which limit their life and social activity. There is an increased risk of sudden falls of patients with a history of cerebral stroke: about a fifth of them falls during subsequent 2–2.5 years following a stroke, and up to a half of such falls can result in serious injuries (Lim et al., 2012). There are sufficiently valid tools for risk assessment of potential falls, for example, Berg Balance Scale (Tilson et al., 2012). A more objective approach involves the use of stabilometric equipment. 40 patients (21 women and 19 men, mean age 61 ± 4 years) with ischemic stroke in the vertebrobasilar area in the early recovery period (21 days–6 months post stroke), underwent a complex technique of balance and stability disorders assessment, including the use of clinical rating scales and stabilometric examination. Compared to the assessment scheme commonly used in clinical practice, which usually

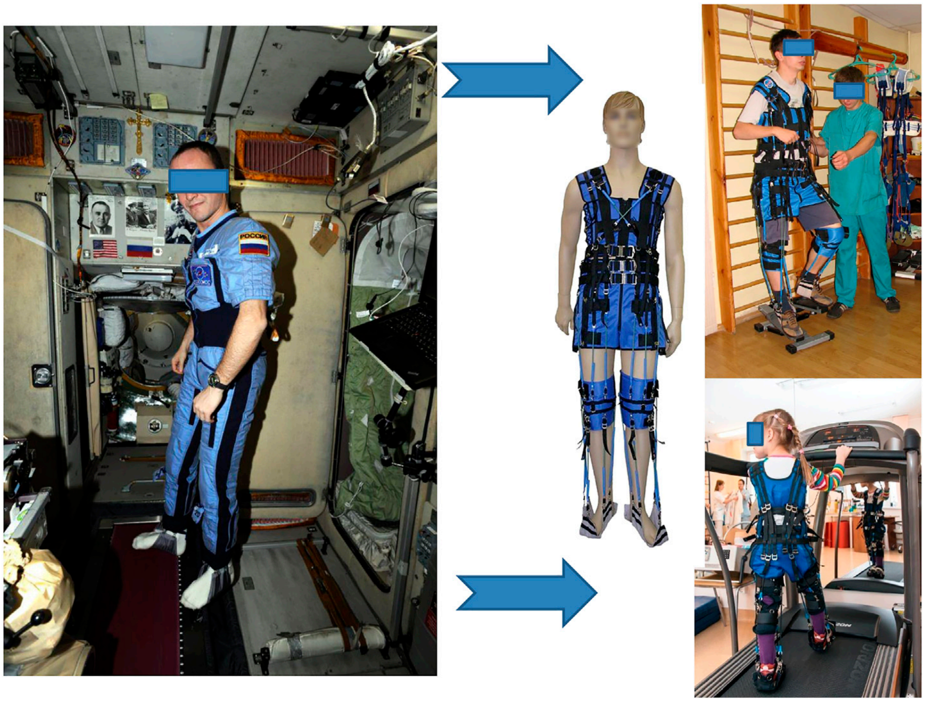

FIGURE 1 | Axial loading suit. On the left—"Penguin" space suit and its application on board the International Space Station; on the right–its clinical version "Regent" (Center of Aerospace Medicine and Technologies, Russia) and its adaptation for rehabilitation of adult patients and children. Photo credit IBMP

includes only non-instrumental approaches, the addition of stabilometric criteria improves the diagnosis of existing disorders and makes it possible to objectively monitor the condition of patients during treatment and rehabilitation. All patients received treatment in accordance with the existing standards. At the same time, 20 patients out of 40 (13 women and seven men, mean age 62 ± 8 years) received additional therapy, including a set of rehabilitation exercises (vestibular and respiratory gymnastics), support reaction biofeedback (based on stabilography), training on a stabilometric platform in an axial loading suit "Regent". The control group (20 patients) was comparable to the main group in all parameters. At the beginning of treatment, average score on the Berg Balance Scale for all patients was 38 points, at the end it was 42 points. In five patients out of 40, the Berg Balance score did not change. It should be noted, that an increase in dynamics for this scale was better observed in those 20 patients who received additional treatment–Berg Balance Scale average score increased from 38 to 46 (by ~19%), and among the remaining 20 patients without additional treatment, it increased from 37 to 39 (by ~5%) (Romanova et al., 2014).

In another study, 28 patients with acute stroke in the vertebrobasilar area, suffering from dizziness, equilibrium and stability disturbances, were randomized into two equal groups (n = 14). The control group underwent traditional pharmacological and physical treatment, while the main group was additionally treated with "Regent" suit. Rehabilitation of patients in acute period of ischemic stroke with "Regent" suit significantly improved their stabilometric parameters (the Romberg's test) and vertical stability assessed with various clinical scales (Bohannon, Perry, Stolyarova, Berg Balance Scale). According to the Berg Balance Scale, vertical stability of the main group patients was 40% ($p<0.001$) higher than in the control group at the end of treatment (Kubriak et al., 2014).

Russian Research Center of Neurology also performed a study investigating the effectiveness of "Regent" axial loading suit in rehabilitation of patients with focal lesions of central nervous system (consequences of acute cerebrovascular disturbances, traumatic brain injuries). 324 patients (197 male and 127 female) with ischemic cerebrovascular pathologies were involved in this study. The control group (100 patients) underwent traditional complex rehabilitative treatment; the main group complex treatment also involved training with "Regent" suit. After the course of rehabilitation there was a more significant decrease in movement disturbances in the main group compared to the control group. Degree of paresis decreased in both groups, however, this change was more significant in the main group (by 33%), than in the control group (by 20%). Muscle spasticity also decreased in both groups: by 18% in the main group and by 15% in the control group. State of bathyesthesia increased in comparison with pre-rehabilitation tests (by 31% in the main group and by 22% in the control group). Average walking speed also increased to a higher extent in the main group (by 33%), than in the control group (by 17%). It is interesting to mention, that the use of "Regent" suit had a positive effect not only on the patients' locomotor patterns, but it also improved their neuropsychological functions (pronunciation and grammar skills correction, active vocabulary enlargement, etc.) (Chernikova, 2016).

3.2 Rehabilitation of Children With Cerebral Palsy

Cerebral spastic infantile paralysis (cerebral palsy, CP) is one of the most common childhood disabilities (Vitrikas, Dalton and Grant, 2020). According to worldwide statistics, cerebral palsy occurs in 2.5 children out of 1000 (Mavlyanova, 2020). There are various clinical features of CP, encompassing a broad range of abnormalities, which mostly include non-progressive movement disorders, poor balance and sensory deficits (Vitrikas, Dalton and Grant, 2020). Different treatment strategies, including physiotherapy, occupational therapy and the use of orthoses are widely applied in mitigation of disabilities, provoked by CP. Along with that, in the last decade, anti-spasticity drugs and orthopaedic surgery were used. The most up-to-date treatment and rehabilitation approach most likely involves the use of therapy garments and orthoses (Romeo et al., 2018). The existing therapy garments include "Adeli" suit, "TheraSuit", full body suit (Kendall-Camp United Kingdom Ltd.), dynamic elastomeric fabric orthosis (DEFO), stabilizing pressure input orthosis (SPIO), "UpSuit",United Kingdom "Second Skin" and "PediaSuit" (Karadağ-Saygı and Giray, 2019).

A single-subject report investigating long-term outcomes of "Adeli" suit treatment (AST) described its high efficiency in improving gait, gross motor function and balance in a child (female, 8 years old) with diplegic cerebral palsy. AST was applied for a total of 50 min, once a week, for 18 weeks. In a 10-m walking speed test, the time of test performance significantly decreased ($p = 0.014$) after AST. It is important to mention, that a notable decrease in test performance time was already observed after the very first AST session (Ko et al., 2015). However, AST usually consists of lengthy treatment sessions, which are more frequent, but the overall treatment program is shorter: they mostly last 5–6 h a day, 6 days a week, for 4–5 weeks (Turner, 2006). In another study, 24 children with CP were treated for 4 weeks (2 h daily, 5 days per week, 20 sessions). The aim of this study was to investigate whether children with CP receiving physical therapy with AST would better improve their motor function and mechanical efficiency than children receiving regular therapy based on neurodevelopmental approach (NDT). Children were matched by age and functional status and randomly assigned in two groups: AST group ($n = 12$; eight males, four females; mean age 8.3years) and NDT group ($n = 12$; nine males, three females; mean age 8.1year). The study specifically revolved around the effects of AST on gross motor function and energy cost quantified by mechanical efficiency. There was a trend for reduction in metabolic cost for a given amount of external work after AST compared with NDT. A significant time effect for gross motor function in all participants after 1 month of intensive physiotherapy treatment was greater than expected from the usually observed in children with CP at that age. These findings support the idea that intensive therapy, either AST or NDT can generally accelerate the acquisition of motor abilities in children with CP. The study also showed that AST can optimize motor skills in children with initially higher level of gross motor skills, as reflected by a reduced metabolic cost of external work (Bar-Haim et al., 2006). Another study of AST approach by K.A. Semenova (1997) reported on 60 children with spastic diplegia and 34 children with hyperkinetic diplegia who underwent AST and traditional treatment. Major improvements were reported with AST approach in comparison with the traditional treatment (protocol not specified), but statistical methods and analysis were unclear (Semenova, 1997).

4 MECHANICAL STIMULATION OF THE SOLES' SUPPORT ZONES FOR NEUROREHABILITATION

In order to mitigate weightlessness-derived movement disorders, scientists at the Institute of Biomedical Problems of the Russian Academy of Sciences together with the VIT company (Russia) developed another unique device "Korvit",United Kingdom a plantar compensator of support unloading (**Figure 2**). "Korvit" allows to reproduce the support stimuli inflow that occur in the process of natural locomotion. The device consists of a control unit, a power supply module, MRI-compatible air ducts, and orthoses with bladders inbuilt into soles that are fixed on the subject's feet. "Korvit" creates pneumo-mechanical pressure on the foot support zones using bladders that operate in real locomotion regimes (75 steps per minute, 40 kPa pressure). These bladders are inbuilt in such a way as to stimulate the feet areas with maximum density of support mechanoreceptors (Fater-Paccini and Meisners' bodies) (Kremneva et al., 2013). Another important "Korvit" feature is that it allows to perform rehabilitation regardless of the patient's immobility degree (Chernikova et al., 2013).

4.1 Rehabilitation of Children With Cerebral Palsy

Proprioceptive stimulation is one of the methods with proven effectiveness in improving motor activity. In children with motor disorders, proprioceptive impulses can normalize to a certain extent the activity of damaged nervous system structures that control motility. It can also slow down and prevent the development of pathological changes in the musculoskeletal system (Prityko et al., 2019). The method has been tested in a study of 87 children (28 girls and 59 boys) with various forms of cerebral palsy aged from 1 to 16 years. Clinical examination of children with spastic diplegia after the course of support stimulation showed a decrease in the severity of tonic reflexes and a decrease in muscle tone, which manifested in positive dynamics of spasticity tests, full foot support acquisition, and absence of leg scissoring. Eight children under the age of two (53%) developed new motor skills: the ability to independently sit down from a supine position, leaning on one arm; to stand and move with walking aid. Five children under the age of four (14.7%) began to walk independently. Children who originally were able to move independently changed the pathological stereotype of walking due to the extinction of primitive tonic reflexes: hip extension increased; the internal rotation of hips and the pathological feet position decreased; foot rolling pattern appeared (rock-up from heel to toe). In patients with the

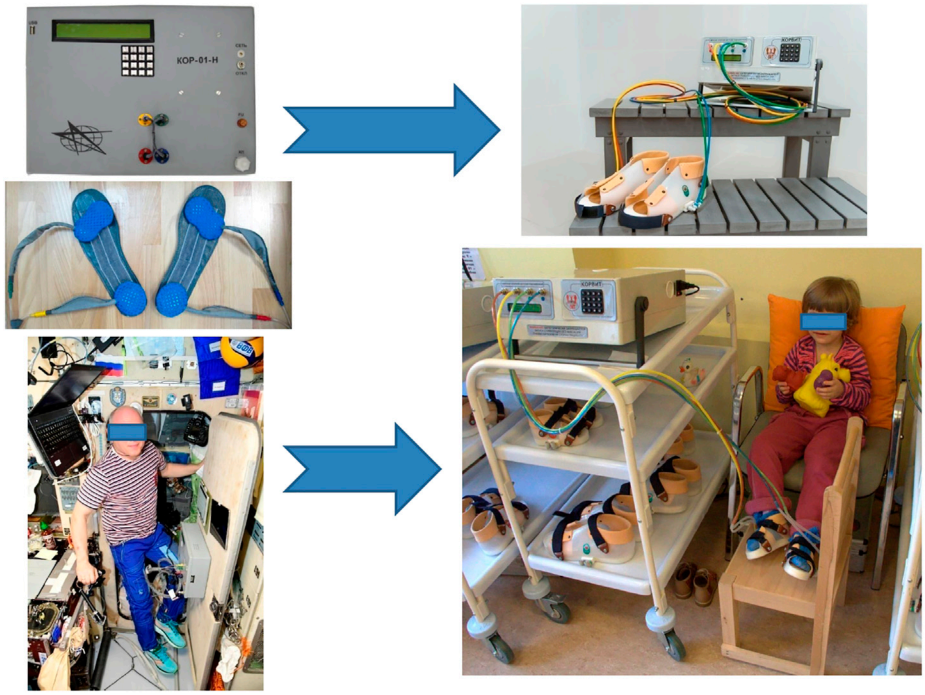

FIGURE 2 | Compensator of support unloading. On the left—space device "KOR" and its application on board the International Space Station; on the right–its clinical version "KORVIT" (Center of Aviaspace Medicine and Technologies, Russia) used for rehabilitation of patients. Photo credit IBMP.

hemiparetic form of cerebral palsy, manifestations of paresis decreased, while the range of motion in the ankle and knee joints of the paretic limb increased. Children could walk a greater distance without visible fatigue, fully loading both feet. In children with the atonic-astatic form of the disease, muscle tone increased, muscle strength and stability increased when walking, coordination of movements improved, manifestations of motor ataxia and motor awkwardness decreased. These results indicate that support stimulation with "Korvit" is a pathogenetically justified, effective and safe method of rehabilitation treatment of children with cerebral palsy. (Levchenkova et al., 2012).

"Korvit" is also successfully used for effective rehabilitation of patients after traumatic brain injuries which caused various movement disorders (Petrova et al., 2019), of patients with Guillain–Barré syndrome (acute sensory-motor polyneuropathy) (Khoroshun, Piradov and Chernikova, 2012) and of children with shin fractures (Serova, Tishchenko and Nikishov, 2012).

4.2 Post-Stroke Rehabilitation

Depending on the stroke stage (acute, sub-acute or chronic), rehabilitation therapists use various techniques and methods to restore patients' damaged functions (Shvarkov et al., 2011; Nordin, Xie and Wünsche, 2014). Rehabilitation measures in acute and sub-acute stroke stages are aimed at reducing functional deficiency and prevention of post-stroke complications. First of all, passive kinesiotherapy (passive movements in all large joints) is performed. It allows to activate the flow of afferent impulses to the perifocal zone of functional asynapsia in the brain, and switch on the motor zones of the cerebral cortex. Another treatment method is verticalization: either passive (with the assistance of verticalizer table), or active. The purpose of passive verticalization is to perform orthostatic training, to preserve afferentation from articular and muscle-tendon receptors and prevent thrombosis in the veins of lower extremities. During this time period, rehabilitation therapists begin proprioceptive stimulation of the soles' support zones with "Korvit" (Shvarkov et al., 2011).

The scientists of Russian Research Center of Neurology proved the clinical effectiveness of the soles' support zones mechanical stimulation. They showed that it normalizes muscle tone in the paretic leg and prevents the development of severe spasticity in the extensors of the foot, as well as contributes to an earlier stance and independent movement skills development. The study included 45 patients aged 61 (55.0; 66.0) years with an average of 1 day (1.0; 2.0) from the onset of moderate and severe stroke. The study group was comprised by 24 patients who received both mechanical stimulation of the plantar support with "Korvit" device (75 steps per minute) and conventional therapy in the first hours of stroke. The control group consisted of 21 patients who received only conventional therapy. All patients on admission and 21 days post-stroke were tested according to international clinical scales (NIHSS, Rankin, Barthel, Fugl-Meyer, Ashworth). It turned out that the inclusion of "Korvit" stimulation in the complex of rehabilitation measures can accelerate the restoration of muscle strength in the paretic leg, improve balance in the sitting and standing positions, and walking. In addition to that, the inclusion of "Korvit" in the early post-stroke rehabilitation program (from the very first day of rehabilitation) improves muscle tone in paralyzed limbs. It can be suggested that "Korvit" stimulation is effective at early stages of rehabilitation after acute stroke primarily due to correction of postural and tonic disorders triggered by functional support deprivation in the gravitational muscles (Glebova, Maksimova and Chernikova, 2014). The obtained results can be explained on the basis of the study by Tomilovskaya Elena (Tomilovskaya et al., 2013), who showed that the use of mechanical stimulation of the soles' support zones performed in the mode of locomotion leads to activation of spinal locomotor structures. In addition, clinical neuroimaging studies, performed in the Russian Research Center of Neurology showed that mechanical stimulation of the soles' support zones in standing and slow walking modes provokes activation of supraspinal structures involved in control of locomotion (primary soma of the sensory cortex, premotor, dorsolateral prefrontal cortex and insular lobules). Simulation of standing was accompanied by greater involvement of the prefrontal cortex. Simulation of slow walking mostly involved sensorimotor parts of the cortex and triggered motor synergies (Kremneva et al., 2012; Glebova, Maksimova and Chernikova, 2014).

5 COMPLEX REHABILITATION THERAPY

Nowadays, some clinicians use complex rehabilitation therapy, which includes both: axial loading suit and soles' support stimulation. Such complex approach was assessed in a 15-year study of 3000 post-stroke patients aged from 20 to 86 years. They were divided into several pathology-dependent groups:

1. 80%—rehabilitating from the consequences of acute violations of cerebral circulation in the early and late recovery periods;
2. 14%—rehabilitating from the consequences of severe traumatic brain injury;
3. 4%—patients rehabilitating after neurosurgical brain surgery;
4. 2%—rehabilitating from spinal strokes and their consequences.

The duration of rehabilitation treatment averaged 21 days. The main clinical manifestation in all groups was deep spastic hemiparesis. Neurorehabilitation activities included individual sessions with rehabilitation therapist, who, in addition to performing massage and exercise therapy, also used axial loading suits and proprioceptive stimulation with "Korvit" device. Clinically significant improvement after the treatment was observed in 61% of patients. Improvement of occupational skills was achieved in 30% of patients, 19% of which returned to their previous work. Recovery of walking skills and everyday life self-care improved in 70% of patients. Walking stereotype improved in 29% of patients. 28% of patients began to walk independently, and in 24% of patients the number of stops due to muscle weakness decreased. There was a significant ($p < 0.05$) increase in muscle strength and a decline in muscle tone in all groups. Therapeutic effects also included a decrease in the degree of upper limbs paresis, an increase in pace frequency and movement volume, and correction of movement coordination. Patients also noted an improvement in nocturnal sleep, overall condition and mood (Shvarkov et al., 2011).

Complex and personalized application of the above listed methods in neurorehabilitation programs makes it possible to effectively treat neurological patients even with significant brain damage by activating plastic processes in the brain. The results obtained by the Russian Research Center of Neurology are already actively used in clinical practice, and in the near future may be included in the rehabilitation programs of Russian clinics and hospitals specializing in restorative medicine (Piradov, Chernikova and Suponeva, 2018).

6 CONCLUSION

Summing up, it can be said that space technologies have made a great contribution not only to space medicine, but they have strongly influenced the development of terrestrial medicine, which actively involves these technologies in everyday practice. Methods of proprioceptive correction are aimed at complex restoration of neurological functions, thus, increasing the effectiveness of rehabilitation treatment of patients suffering from severe motor disturbances, triggered by children cerebral palsy, ischemic stroke, traumatic brain injuries and other spinal or cardiovascular pathologies. Their application helps to recover disturbed motor stereotype and prevent maladjustment to everyday life. In addition to that, inclusion of these means in early rehabilitation programs enhances the rehabilitation process and makes it more effective. Complex application of proprioceptive correction methods in neurorehabilitation programs makes it possible to effectively treat neurological patients even with significant brain damage. However, it is clear that these methods should only be applied as additional therapy along with conventional rehabilitation techniques. Their isolated administration would most likely be ineffective, especially in patients with severely damaged motor functions. The severity of motor disturbances is also a limiting factor for application of axial loading suit, as it cannot be used for patients who are paraplegic.

AUTHOR CONTRIBUTIONS

EM wrote the draft of the manuscript and made its revisions. MB prepared the part on axial loading suit. IR and AS contributed in the global revision and reorganizing of the manuscript. TS prepared the part on support stimulation. ET made the final revision of the manuscript and prepared the part of Regent usage in clinics.

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Introducing the Concept of Exercise Holidays for Human Spaceflight - What Can We Learn From the Recovery of Bed Rest Passive Control Groups

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In an attempt to counteract microgravity-induced deconditioning during spaceflight, exercise has been performed in various forms on the International Space Station (ISS). Despite significant consumption of time and resources by daily exercise, including around one third of astronauts' energy expenditure, deconditioning—to variable extents—are observed. However, in future Artemis/Lunar Gateway missions, greater constraints will mean that the current high volume and diversity of ISS in-flight exercise will be impractical. Thus, investigating both more *effective* and *efficient* multi-systems countermeasure approaches taking into account the novel mission profiles and the associated health and safety risks will be required, while also reducing resource requirements. One potential approach is to reduce mission exercise volume by the introduction of exercise-free periods, or "*exercise holidays*". Thus, we hypothesise that by evaluating the 'recovery' of the no-intervention control group of head-down-tilt bed rest (HDTBR) campaigns of differing durations, we may be able to define the relationship between unloading duration and the dynamics of functional recovery—of interest to future spaceflight operations within and beyond Low Earth Orbit (LEO)—including preliminary evaluation of the concept of exercise holidays. Hence, the aim of this literature study is to collect and investigate the post-HDTBR recovery dynamics of current operationally relevant anthropometric outcomes and physiological systems (skeletal, muscular, and cardiovascular) of the passive control groups of HDTBR campaigns, mimicking a period of 'exercise holidays', thereby providing a preliminary evaluation of the concept of 'exercise holidays' for spaceflight, within and beyond LEO. The main findings were that, although a high degree of paucity and inconsistency of reported recovery data is present within the 18 included studies, data suggests that recovery of current operationally relevant outcomes following HDTBR without exercise—and even without targeted rehabilitation during the recovery period—could be timely and does not lead to persistent decrements differing from those experienced following spaceflight. Thus, evaluation of potential exercise holidays concepts within future HDTBR campaigns is warranted, filling current knowledge gaps prior to its potential implementation in human spaceflight exploration missions.

Keywords: microgravity, spaceflight, deconditioning, astronaut, countermeasures

1 INTRODUCTION

Spaceflight is associated with anthropometric adaptations such as loss of body mass (Matsumoto et al., 2011), stature increments (Green and Scott, 2018) and deconditioning of physiological systems including musculoskeletal (Trappe et al., 2009; Stavnichuk et al., 2020) and cardiopulmonary deconditioning (Charles and Lathers, 1991; Hargens and Richardson, 2009). To counteract microgravity-induced deconditioning, exercise in various forms has been performed since early space missions and has evolved significantly over the years (Hayes, 2015; Scott et al., 2019). Current exercise prescriptions for ESA astronauts on-board the International Space Station (ISS) consist of approximately 90 min concurrent aerobic and resistive exercise training per day throughout long-duration missions, involving use of a resistive exercise device (ARED), a treadmill (T2) and a cycle ergometer (CEVIS) (Petersen et al., 2016). As a result, around one third of the astronauts' daily energy expenditure is spent on exercise (Laurens et al., 2019; Scott et al., 2020). Despite this, multi-system physiological deconditioning—albeit to variable extents—is still observed in most long-duration ISS crew (Weber et al., 2020; Scott et al., 2021).

With entirely new mission profiles on the horizon (e.g., Artemis and Lunar Gateway), where microgravity exposure will be significantly shorter, but where crew will be exposed to Lunar hypogravity upon landing on the Lunar surface (NASA, 2020), needs and requirements for in-flight exercise countermeasures will likely change significantly, driven by the novel mission profiles and associated health and safety risks. This could imply that for Lunar gateway missions with Lunar surface EVAs after prolonged (30–90 days) exposure to microgravity in Lunar orbit, primary needs and requirements of the countermeasure programmes may not need to focus on maintaining bone mineral density, muscle strength and VO2max as is the case in current long-duration mission profiles. However, a unique and critical period in these missions will be the transition from prolonged exposure to microgravity, to hypogravity on the Lunar surface. Both Miller et al. (2018) and Mulavara et al. (2018) reported significantly worse performances of functional tasks (e.g., seat egress and walk, recovery from fall, jump down) and sensorimotor tests (e.g., dynamic posturography, tandem walk) following long-duration spaceflight with extensive daily exercise regimens. Thus, recovery of orthostatic tolerance, postural stability, spatial orientation, and balance will likely be of greater importance to assure crew safety and mission success as is currently the case. Therefore, definition of future in-flight countermeasure programmes will most likely benefit from shifting the focus from current operationally relevant parameters for long-duration spaceflight (i.e., skeletal, muscular, and cardiovascular) to those more relevant to the new mission profiles involving Lunar surface EVAs. Additionally, vehicle constraints will also mean that the currently prescribed high volume-high load exercise with a great energy expenditure and diversity of ISS in-flight exercise currently prescribed might not be appropriate (Laurens et al., 2019). Optimization of exercise programmes could also reduce the metabolic cost, and thus associated energy expenditure, thereby reducing food, water and respiratory gas (i.e., oxygen provision and carbon dioxide removal) requirements, which would be highly advantageous since re-supply opportunities will be greatly reduced, or impossible (Drake et al., 2010). One potential approach to reduce overall exercise volume and associated energy expenditure is the introduction of exercise-free periods, or "*exercise holidays*", throughout—a part of—the duration of the space mission.

Exercise holidays are commonly prescribed to elite athletes, including offseason breaks as part of training periodization that seeks to facilitate optimal performance during specific periods (Lorenz et al., 2010). During periodization, training variables such as type, load, sets and within set repetitions are manipulated to maximize appropriate training adaptations, whilst attempting to minimize excessive fatigue, and or injury risk (Buford et al., 2007; Lorenz et al., 2010). Hence, athletes may be prescribed periods where exercise volume and intensity are significantly reduced or even minimal (Lorenz et al., 2010).

Translating this to the context of spaceflight, crewmembers would thus be prescribed periods without in-flight exercise countermeasures—increasing the time to be spent on scientific research, maintenance, or extravehicular activities—and periods with in-flight exercise countermeasures, tasked to optimize functionality in-flight, during landing or the immediate post-flight period. However, they do not seek to optimize athletic performance, but rather maintain health, wellbeing and functionality, in particular upon landing when astronauts are exposed to hypogravity on the Lunar surface, or re-exposed to Earth's gravity in a state of microgravity-induced deconditioning.

In fact, astronaut gravitational unloading is more akin to bed-bound patients, such as those admitted to intensive care. Such patients experience rapid and profound musculoskeletal (Puthucheary et al., 2010) and cardiopulmonary deconditioning (Benington et al., 2012) leading to a protracted impairment of everyday activities (Svenningsen et al., 2017). As a result, intensive rehabilitation is required to promote performance of everyday activities, resumption of independence and the improvement of quality of life (Denehy and Elliott, 2012; Svenningsen et al., 2017).

The most commonly employed ground-based analogue is long term six-degree head-down-tilt bed rest (HDTBR) which mimics many of the physiological effects associated with long-duration space missions (Hargens and Vico, 2016). HDTBR studies have the advantage of being able to study larger sample sizes, allow better standardisation (e.g., fixed daily routine for all participants), and to minimise some of the potential confounding factors associated with spaceflight (e.g., space radiation) (Kakurin et al., 1976; Regnard et al., 2001; Winnard et al., 2017). HDTBR studies of differing durations have been performed, reporting broadly similar changes in anthropometric (e.g., mass loss (Matsumoto et al., 2011)), skeletal (e.g., reduced bone mineral density (Baecker et al., 2003) and altered bone architecture (Spector et al., 2009)), muscular (e.g., loss of muscle mass (Droppert, 1993)), and cardiovascular parameters (e.g., reduced cardiac output (Arbeille et al., 2001)), as those observed following spaceflight. As a result, HDTBR participants also require a period of rehabilitation (Winnard et al., 2019).

Thus, improving the understanding of induced de-conditioning, but mainly the dynamics of recovery of passive control groups of HDTBR campaigns is essential. Such knowledge is critical for defining, evaluating, and optimizing in-flight exercise countermeasure prescriptions of future space exploration missions, but may also facilitate evaluation of the concept of exercise holidays.

However, to this date, the post-HDTBR recovery period has received relatively little attention. In fact, despite numerous HDTBR studies being performed, there is still no agreement on the approach to rehabilitation (Winnard et al., 2017). Furthermore, very few HDTBR participants have received an individualized rehabilitation programme similar to that provided to astronauts (Petersen et al., 2016, Petersen et al., 2017). Indeed, the lack of attention paid to the post-HDTBR period was highlighted by Greenleaf and Quach (2003), having reviewed, at that time, 157 published HDTBR studies. Greenleaf and Quach also highlighted a single study that evaluated an exercise protocol consisting of supine treadmill walking and a cycle ergometer that was instigated at day 140 of a HDTBR study, reporting that various musculoskeletal and cardiovascular parameters returned to baseline by day 240 of continuing HDTBR (Grigoriev et al., 1992). This data suggests that the concept of an exercise holiday may hold promise—but is insufficient on its own. To further explore this, we hypothesise that by evaluating the recovery of the passive control groups of HDTBR campaigns of differing durations we may be able to gain insights into the dynamics of functional recovery following a period of simulated exercise holidays.

Thus, the aim of this literature study is to, for the first time, collect and investigate the post-HDTBR recovery dynamics of current operationally relevant anthropometric outcomes and physiological systems (skeletal, muscular, and cardiovascular) of the passive control groups of HDTBR campaigns, mimicking a period of exercise holidays, thereby providing a preliminary evaluation of the concept of 'exercise holidays' for spaceflight, within and beyond Low Earth Orbit (LEO).

2 MATERIALS AND METHODS

2.1 Data Sources and Searches

An initial systematic search was performed based on that reported by (Fiebig et al., 2018) that used Boolean search strings based on three overarching categories ("microgravity", "countermeasures", and "operationally relevant outcome parameters"), as defined by ESA's Space Medicine Team. This search, performed on 18 June 2021, evaluated the various databases: Pubmed, Web of Science, Cochrane Collaboration Library, Institute of Electrical and Electronics Engineers database as well as ESA's "Erasmus", the National Aeronautics and Space Administration's (NASA) "Life Science Data Archive" and "Technical Reports Server" and the German Aerospace Centre's (DLR) database "elib" for relevant studies published in English.

Additionally, a second search was performed on 7 July 2021 in Pubmed only, based on the "microgravity" and "operationally relevant outcome parameters" categories to ensure no studies were excluded that did not include a countermeasure intervention.

Results of both searches were combined, and duplicate records removed to yield a single file used for study selection (see ***Supplementary Table S1***).

2.2 Study Selection

Relevant studies were identified using predefined selection criteria according to the Population Intervention Comparison Outcomes Study design (PICOS) methods:

1) **Population—**Healthy adult female and/or male bed rest participants (≥18 years old).
2) **Interventions—**Studies utilizing six-degree head-down tilt bed rest with a minimum duration of 5 days—in accordance with the categories for bed rest study duration described by Sundblad and Orlov (2014)—and at least two follow-up evaluations during the post-bed rest (recovery) period.
3) **Control Conditions—**Only bed rest participants that were assigned to a passive/no intervention/placebo control condition were included in this review. Data from participants assigned to an exercise, nutrition, or any other intervention were not extracted.
4) **Outcomes**—Only studies containing outcomes considered to be "operationally relevant" were included. Outcome parameters within the categories of interest (see below) were defined as "operationally relevant" by members of ESA's Space Medicine Team who performed a scoping exercise based on parameters reported in papers extracted by Fiebig et al. (2018) where relevance was defined as:
5) "Parameters having a direct impact on physical performance in space and after landing, and/or that would jeopardise nominal mission performance when deteriorated."
6) **Study Designs**—Randomised controlled trials (RCT) and controlled clinical trials (CT) were included.

Phase 1 involved several independent reviewers (RE, TW, NH, RHL, DG) independently (blinded) applying the selection criteria on titles and abstracts via the Rayyan Web Application (Ouzzani et al., 2016). Phase 2 involved blinded screening of the full-text resources, based on the same pre-defined selection criteria.

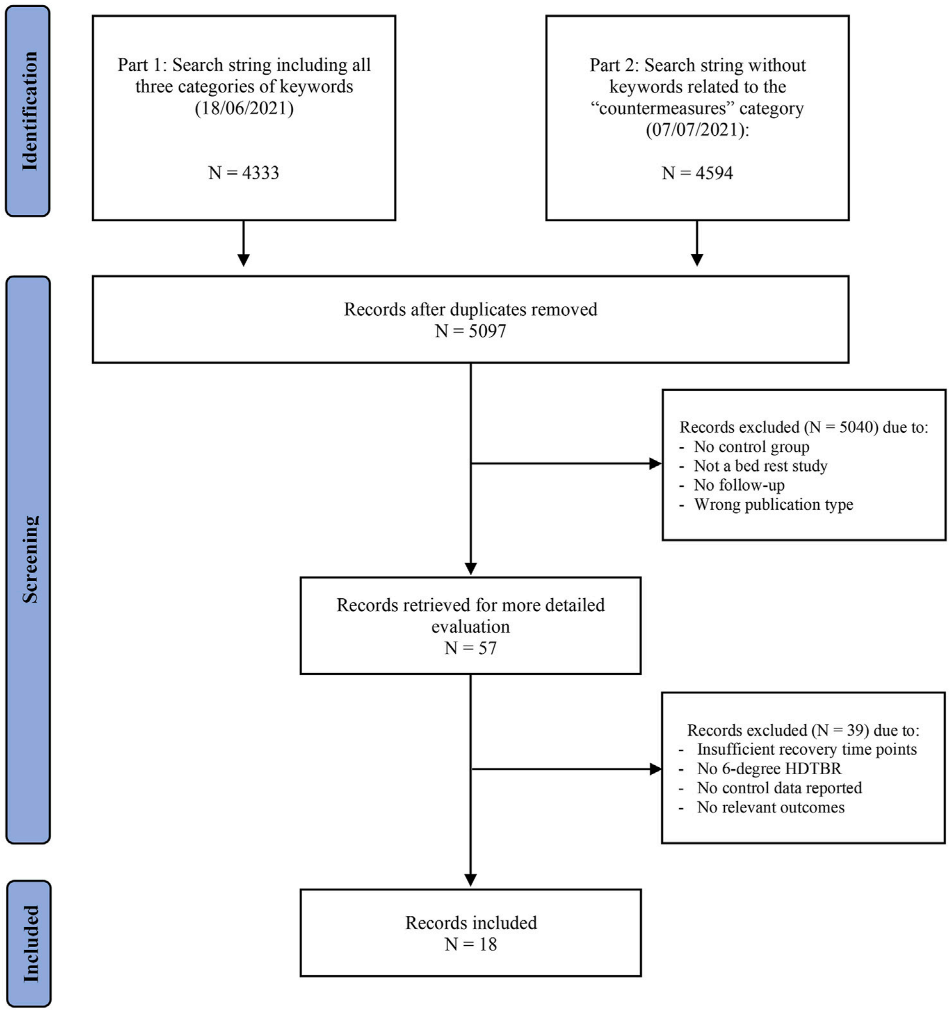

FIGURE 1 | Flowchart of the selection process.

2.3 Data Extraction and Quality Assessment

2.3.1 Data Extraction

If the study was eligible, the following data were extracted:

1) **General Population Characteristics**—Number of participants, sex distribution, mean, standard deviation (SD) and range of age (years), body height (centimetres) and body mass (kg).
2) **Characteristics of the Six Degree Head Down Bed Rest Intervention—**Number of bed rest days, diet, daily routine, standardization of bed-rest phases (e.g., same baseline data collection, same bed-rest time), sunlight exposure.
3) **Characteristics of the Recovery Period**—Number of days of follow-up, time-points of measurements during recovery period, standardization of recovery period (e.g., controlled recovery phases and conditions).
4) **Reported Outcome Parameters—**Numeric values (Mean and SD/standard error (SE); Median and Interquartile Range; % change from baseline with SD) for each relevant parameter at baseline and at each time-point during recovery were extracted. Each parameter was classified under one of the following categories: “Anthropometric Outcomes”, “Skeletal System”, “Muscular System” and “Cardiovascular System”. For a full overview of all extracted parameters, see ***Supplementary Material 1****—Operationally Relevant Outcome Parameters.*

As adaptation of physiological s-ystems during the recovery period were largely of secondary importance in the majority of included HDTBR studies, recovery data was extrapolated where appropriate from tables and figures. Extrapolation of data from figures was performed with WebPlotDigitizer (version 4.5; California, United States) software, which has been shown to yield reliable and valid data (Drevon et al., 2016).

2.3.2 Quality Assessment

Quality appraisal of the methodology of the included bed rest studies was assessed using the AMSRG tool (Winnard and

TABLE 1 | Quality appraisal of bed rest method to simulate microgravity.

Author	Number of BR days	6° Head down Tilt	Individualized and Controlled Diet	Set Daily Routine with Fixed Wake/Sleep Time	BR Phases Standardized for all Participants	Uninterrupted BR except for Test Condition	Sunlight Exposure Prohibited	All Measurements Taken Same day and Time	*Total score*
Short duration HDTBR (5—14 days)									
Beck et al. (1992)	10	Y	Y	?	Y	Y	?	Y	6
Rittweger et al. (2015)	5	Y	Y	Y	Y	Y	?	Y	7
Samel et al. (1993)	7	Y	Y	Y	Y	Y	?	Y	7
Schulz et al. (1992)	10	Y	Y	?	Y	Y	?	Y	6
Stegemann et al. (1985)	7	Y	?	?	Y	Y	?	Y	5
Medium duration HDTBR (15—59 days)									
Convertino et al. (1990)	30	Y	Y	Y	Y	Y	?	Y	7
Ferretti et al. (2001)	42	Y	?	?	Y	Y	?	Y	5
Long duration HDTBR (≥60 days)									
Alkner and Tesch (2004)	90	Y	?	?	Y	?	?	Y	4
Alkner et al. (2016)	90	Y	?	?	Y	?	?	Y	4
Belavý et al. (2011a)	60	Y	Y	Y	Y	Y	?	Y	7
Belavý et al. (2017)	90	Y	Y	?	Y	Y	?	Y	6
Beller et al. (2011)	60	Y	Y	?	Y	Y	?	Y	6
Kramer et al. (2017)	60	Y	Y	Y	Y	Y	?	Y	7
Linnarsson et al. (2006)	120	Y	?	?	Y	Y	?	Y	5
Liu et al. (2015)	60	Y	Y	?	Y	N	?	Y	5
Rittweger et al. (2007)	90	Y	Y	?	Y	Y	?	Y	6
Rittweger and Felsenberg (2009)	90	Y	Y	?	Y	Y	?	Y	6
Westby et al. (2016)	60	Y	Y	?	Y	Y	?	Y	6
Average									5.8
SD									1.0

Note. *This tool allows to assess how well bed rest studies have been conducted to simulate actual human spaceflight developed by (Winnard et al., 2017; Winnard and Nasser, 2017). The higher the total score, the better the quality and the greater the transferability to human spaceflight; BR: bed rest; Y: yes, criteria is met; N: no, criteria is not met; ? Unclear/information is lacking.*

Nasser, 2017). This purpose-built tool uses eight criteria to detail how similar the conditions of the ground-based analogue are compared with actual spaceflight, thereby assessing the ability to simulate the physiological effects of a prolonged exposure to microgravity: 1) Number of bed rest days stated; 2) six degrees head down tilt; 3) individualized and controlled diet; 4) set daily routine with fixed wake/sleep time; 5) bed rest phases standardised for all participants; 6) uninterrupted bed rest except for test condition; 7) sunlight exposure prohibited; 8) all measures taken at the same day and time.

Each study was assessed against each criterion, whether it was met "Y"; not met "N"; or whether it was unclear/information was lacking "?". All criteria which were met are ascribed a value of 1 and summed to yield a total score: ranging from 0 (poor) to 8 (excellent).

TABLE 2 | Characteristics of the individual studies.

Author	Bedrest campaign	# Days bed rest	# Days recovery period	Study Sample Characteristics									Space agencies involved	Location - setting
				# Subjects	*# Females*	*Age (years)*			*Body length (cm)*		*Body Weight (kg)*			
						Mean	SD	Min-Max	Mean	SD	Means	SD		
Short duration HDTBR (5—14 days)														
Beck et al. (1992)	HDT′88 study	10	8	6	0	26	4.4	21–34	176	5	72	12.4	DLR, NASA	Germany - DLR
Rittweger et al. (2015)	BRAG1 study	5	5	11	0	34	7	22–42	179	7	76	6	ESA	France - MEDES Facilities
Samel et al. (1993)		7	2	8	0	23.9	2	21–27					DLR	Germany - DLR
Schulz et al. (1992)	HDT′88 study	10	8	6	0	26	4.4	21–34	176	5	72	12.4	DLR, NASA	Germany - DLR
Stegemann et al. (1985)		7	5	6	0	23.3	2.81	20–28	180.7	4.97	73.5	7.6		Germany
Medium duration HDTBR (15—59 days)														
Convertino et al. (1990)		30	30	11	0	38	6.6	30–45	179	2	79	2	NASA	US - NASA-Ames Research Center Human Research Facility
Ferretti et al. (2001)	HDT 94 BR project	42	48	7	0	28	1		176	1	74.7	8.8	ESA	France - MEDES Facilities
Long duration HDTBR (≥60 days)														
Alkner and Tesch (2004)		90	11	9	0	32	4		173	3	72	5		France - MEDES Facilities
Alkner et al. (2016)		90	11	9	0	32	4		173	3	72	5		France - MEDES Facilities
Belavý et al. (2011a)	2nd Berlin Bed Rest Study	60	90	9	0	33.1	7.8		181.3	6	80.6	5.2	ESA, DLR	Germany - Charite Campus Bejamin Franklin (Berlin)
Belavý et al. (2017)	LTBR study	90	360	16	0	32.5	3.4		174	4	70.3	6.1	ESA, CNES, NASDA	France - MEDES Facilities
Beller et al. (2011)	WISE-2005	60	20	8	8	34.4	3.8		162.8	6.2	56.5	3.3	ESA, NASA, CSA, DLR, CNES	France - MEDES Facilities
Kramer et al. (2017)	Cologne RSL study	60	15	11	0	28	6		181	5	76	8	ESA, DLR	Germany - Envihab facility (DLR)
Linnarsson et al. (2006)		120	15	6	0	31		23–42	181		80		ESA	Russia - Institute for Biomedical Problems, Moscow
Liu et al. (2015)		60	15	14	0	30	1		169	1				China - Bed Rest Study Lab - China Astronaut

(Continued on following page)

TABLE 2 | (*Continued*) Characteristics of the individual studies.

Author	Bedrest campaign	# Days bed rest	# Days recovery period	Study Sample Characteristics									Space agencies involved	Location - setting
				# Subjects	***# Females***	***Age (years)***			***Body length (cm)***		***Body Weight (kg)***			
														Research and Training Center
Rittweger et al. (2007)	LTBR study	90	180	16	0	32.5	3.4		174.2	3.9	71.4	6.7	ESA, CNES, NASDA	France - MEDES Facilities
Rittweger and Felsenberg (2009)	LTBR study	90	360	9	0	31.9	3.6	26–37	173.4	3	71.7	5.4	ESA, CNES, NASDA	France - MEDES Facilities
Westby et al. (2016)		60	14	7	3	36	8				72.5	2.8	NASA	US - Flight Analog Research Unit

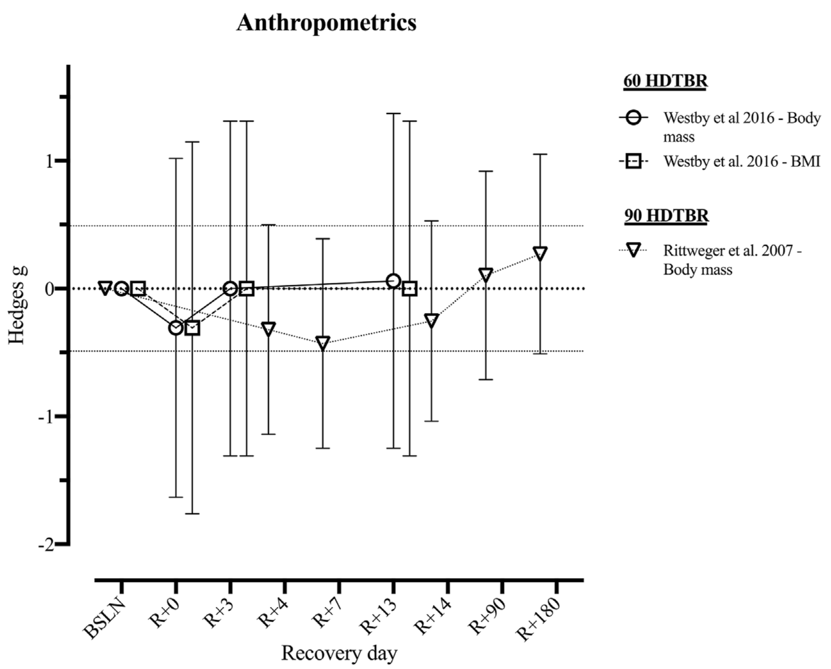

FIGURE 2 | Visualisation of the recovery of outcomes related to '*Anthropometrics Outcomes*' after a period of head-down-tilt bed rest, displayed as Hedges *g* with 95% Confidence Interval. To determine whether a particular outcome could be deemed as "recovered" during the recorded recovery period, the Westlake's Confidence Interval Procedure (Seaman and Serlin, 1998) was used. This procedure tests for equivalence between two means using a confidence interval. To do so, Upper (0.49) and Lower (−0.49) equivalency bounds of interest were determined, corresponding to the limit of a small effect size. When combined with the 95% Confidence Interval of the Hedges *g*, three scenarios are possible: 1) *No evidence of recovery*: It cannot be concluded that the difference between means is trivial as the 95% Confidence Interval falls completely outside the set equivalency bounds; 2) *Weak evidence of recovery*: The results are inconclusive as the 95% Confidence Interval partially falls within the set equivalency bounds, thus including both trivial and non-trivial differences. The dotted lines at 0.49 and −0.49 represent the upper and lower equivalency bounds; 3) *Strong evidence of recovery:* There is practical equivalence as the 95% Confidence Interval falls completely within set equivalency bounds; 60 HDTBR: Included studies implementing a head-down-tilt bed rest period of 60 days (Westby et al., 2016); 90 HDTBR: Included studies implementing a head-down-tilt bed rest period of 90 days (Rittweger et al., 2007); R+…: Respective recovery day; BMI: Body Mass Index.

TABLE 3 | % Change with SD of outcome parameters related to *'Anthropometric Outcomes'*.

Recovery timepoint	Author	Returned to baseline? = Y
	Kramer et al. (2017)	
	60 days HDTBR	
Body Mass		
R+7	−2.23 (1.88)[a]	
Returned to Baseline? (Y/N)	N	0/1
R+14	−1.80 (1.69)	
Returned to Baseline? (Y/N)	N	0/1
Fat Mass		
R+7	0.34 (8.80) [a]	
Returned to Baseline? (Y/N)	N	0/1
R+14	0.17 (7.92) [a]	
Returned to Baseline? (Y/N)	N	0/1
Lean Mass		
R+7	−2.55 (3.10) [a]	
Returned to Baseline? (Y/N)	N	0/1
R+14	−1.47 (2.64) [a]	
Returned to Baseline? (Y/N)	N	0/1

Notes. *data of outcome parameters related to anthropometric outcomes, reported as % change from baseline were extracted and displayed without any alterations for each reported recovery timepoint following a period of 6-degree-head-down-tilt bed rest. For each recovery timepoint the effect was categorize as "Returned to baseline" or "Not returned to baseline". Returned to Baseline? = Y: Whenever the mean % change equals 0% or reverts from + to—/– to +; Returned to Baseline? = N: Whenever the mean % change remains + or –.All values are displayed as Mean % Change from Baseline (SD); HDTBR: 6-degree-head-down-tilt bed rest; Y: yes; N: no.*
[a]*Data extracted from figure using WebPlotDigitizer.*

2.4 Data Analysis

To determine whether an outcome parameter recovered following the HDTBR period, standardized mean differences (Hedges *g*; mean and 95% confidence interval) (Cumming, 2012; Lakens, 2013) were calculated from the reported raw pre- and post-HDTBR mean and SD values (***Supplementary Material 2****—Statistical Calculations*).

A given parameter was deemed to have "recovered" during the recovery period according to the Westlake's Confidence Interval procedure (Seaman and Serlin, 1998) which evaluates mean equivalence using a confidence interval (e.g., 95% CI of Hedges *g*) for the difference between two means. Upper (0.49) and lower (-0.49) equivalency bounds of interest, corresponding to the limit of a small effect size (Sawilowsky, 2009), were then determined. When combined with the 95% CI of the calculated Hedges *g*, three scenarios are possible (Seaman and Serlin, 1998):

1) The 95% CI falls completely outside the set equivalency bounds, thus it cannot be concluded that the difference between means is trivial, hence no evidence of recovery is observed.
2) The 95% CI falls partially within the set equivalency bounds, the results are inconclusive as the 95% CI included both trivial and non-trivial mean differences, hence weak evidence of recovery is observed.
3) The 95% CI falls completely within the set equivalency bounds, the 95% CI reveals a trivial difference, there is practical equivalence, hence strong evidence of recovery is observed.

Using the Confidence Interval approach has two main advantages (Quertemont, 2011). Firstly, the underlying reasoning is easy to understand, i.e., if both limits of the 95% CI are within the predetermined threshold values it can be concluded that there is no effect of practical importance. Secondly, there is no need to agree on a precise value of the threshold for a minimal effect size allowing interpretation of whether the interval limits are sufficiently narrow to be of no practical significance.

If only the percentage change from baseline data was reported for the recovery time-points, values could not be transformed into raw mean and SDs and were thus excluded from Hedges *g* calculations. In this case, vote counting based on direction of effects was used to synthesize such results (Higgins et al., 2021). For each study, the effect was categorized as 'Returned to Baseline' or 'Not Returned to Baseline'. An outcome was deemed to have 'Returned to Baseline' whenever the Mean % Change from Baseline was equal to 0%, or when a negative Mean % Change became positive, or vice versa. An outcome was deemed to have 'Not Returned to Baseline' when the Mean % Change from Baseline remained negative, or positive. The number of effects that Returned to Baseline were then compared with the number that was deemed to have Not Returned to Baseline and were synthesized as the ratio between the number of effects that were deemed to have Returned to Baseline, and the total number of effects reported for that particular outcome.

3 RESULTS

The initial search query generated 5,097 unique hits. After screening, 18 studies (Stegemann et al., 1985; Convertino et al., 1990; Beck et al., 1992; Schulz et al., 1992; Samel et al., 1993; Ferretti et al., 2001; Alkner and Tesch, 2004; Linnarsson et al., 2006; Rittweger et al., 2007; Rittweger and Felsenberg, 2009; Belavý et al., 2011a; Beller et al., 2011; Liu et al., 2015; Rittweger et al., 2015; Alkner et al., 2016; Westby et al., 2016; Belavý et al., 2017; Kramer et al., 2017) met the selection criteria from which data were extracted (**Figure 1**).

3.1 Quality Assessment—Bed Rest Methodology

Total AMSRG scores of the included studies ranged between 4 and 7 out of 8 with a mean score of 5.8 ± 1.0 (**Table 1**). All included studies failed to provide clarity on whether sunlight exposure was prohibited and whether participants were supplemented with vitamin D. Only five studies (36%) (Convertino et al., 1990; Samel et al., 1993; Belavý et al., 2011a; Rittweger et al., 2015; Kramer et al., 2017) reported whether the daily routine was fixed, additionally, information regarding individualized and controlled diet was absent in five (35%) (Stegemann et al., 1985; Ferretti et al., 2001; Alkner and

TABLE 4 | % Change with SD of outcome parameters related to the *'Skeletal System'*.

Recovery timepoint	Author			Returned to baseline? = Y
	Belavý et al. (2011a)	Beller et al. (2011)	Rittweger and Felsenberg (2009)	
	60 days HDTBR	***60 days HDTBR***	***90 days HDTBR***	
Lumbar Spine (L1-L4) BMC				
R+3	−1.30 (0.77)			
Returned to Baseline? (Y/N)	N			0/1
R+14	0.52 (0.85)			
Returned to Baseline? (Y/N)	Y			1/1
R+30	1.77 (0.87)			
Returned to Baseline? (Y/N)	Y			1/1
R+90	0.42 (0.89)			
Returned to Baseline? (Y/N)	Y			1/1
Total Body BMC				
R+3	−0.44 (0.25)			
Returned to Baseline? (Y/N)	N			0/1
R+14	−0.86 (0.25)			
Returned to Baseline? (Y/N)	N			0/1
R+30	−0.63 (0.21)			
Returned to Baseline? (Y/N)	N			0/1
R+90	−0.41 (0.19)			
Returned to Baseline? (Y/N)	N			0/1
Legs BMC				
R+3	−2.35 (0.43)			
Returned to Baseline? (Y/N)	N			0/1
R+14	−2.87 (0.38)			
Returned to Baseline? (Y/N)	N			0/1
R+30	−2.49 (0.40)			
Returned to Baseline? (Y/N)	N			0/1
R+90	−1.27 (0.47)			
Returned to Baseline? (Y/N)	N			0/1
Trunk BMC				
R+3	1.86 (0.77)			
Returned to Baseline? (Y/N)	N			0/1
R+14	1.87 (0.77)			
Returned to Baseline? (Y/N)	N			0/1
R+30	1.29 (0.83)			
Returned to Baseline? (Y/N)	N			0/1
R+90	0.80 (0.87)			
Returned to Baseline? (Y/N)	N			0/1
Tibia (4%) BMC				
R+14			−6.03 (1.64) [a]	
Returned to Baseline? (Y/N)			N	0/1
R+90			−2.95 (0.70) [a]	
Returned to Baseline? (Y/N)			N	0/1
R+180			−1.93 (0.49) [a]	
Returned to Baseline? (Y/N)			N	0/1
R+360			−0.95 (0.42) [a]	
Returned to Baseline? (Y/N)			N	0/1
Tibia (66%) BMC				
R+3	-2.07 (0.52) [a]			
Returned to Baseline? (Y/N)	N			0/1
R+14			−1.97 (0.41) [a]	
Returned to Baseline? (Y/N)			N	0/1
R+15	−2.10 (0.56) [a]			
Returned to Baseline? (Y/N)	N			0/1
R+30	−2.23 (0.62) [a]			
Returned to Baseline? (Y/N)	N			0/1
R+90	−1.59 (0.63) [a]		−0.74 (0.24) [a]	

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TABLE 4 | (*Continued*) % Change with SD of outcome parameters related to the *'Skeletal System'*.

Recovery timepoint	Author			Returned to baseline? = Y
	Belavý et al. (2011a)	Beller et al. (2011)	Rittweger and Felsenberg (2009)	
	60 days HDTBR	*60 days HDTBR*	*90 days HDTBR*	
Returned to Baseline? (Y/N)	N		N	0/2
R+180			–0.11 (0.19) [a]	
Returned to Baseline? (Y/N)			N	0/1
R+360			0.14 (0.12) [a]	
Returned to Baseline? (Y/N)			Y	1/1
Tibia (4%) BMD				
R+3		–3.13 (0.86)[b]		
Returned to Baseline? (Y/N)		N		0/1
R+90		–1.89 (0.78)[b]		
Returned to Baseline? (Y/N)		N		0/1
R+180		–1.65 (0.79)[b]		
Returned to Baseline? (Y/N)		N		0/1
R+360		–1.81 (0.82)[b]		
Returned to Baseline? (Y/N)		N		0/1
Hip BMD				
R+3		–3.50 (0.55)[b]		
Returned to Baseline? (Y/N)		N		0/1
R+45		–2.80 (0.77)[b]		
Returned to Baseline? (Y/N)		N		0/1
R+90		–2.46 (0.66)[b]		
Returned to Baseline? (Y/N)		N		0/1
R+180		–2.03 (0.94)[b]		
Returned to Baseline? (Y/N)		N		0/1
R+360		–0.52 (0.65)[b]		
Returned to Baseline? (Y/N)		N		0/1
Lumbar Spine (L1-L4) BMD				
R+3		0.44 (0.85)[b]		
Returned to Baseline? (Y/N)		N		0/1
R+45		0.92 (0.82)[b]		
Returned to Baseline? (Y/N)		N		0/1
R+90		0.14 (0.75)[b]		
Returned to Baseline? (Y/N)		N		0/1
R+180		0.74 (0.69)[b]		
Returned to Baseline? (Y/N)		N		0/1
R+360		–0.15 (0.77)[b]		
Returned to Baseline? (Y/N)		Y		1/1

Notes. *data of outcome parameters related to skeletal system, reported as % change from baseline were extracted and displayed without any alterations for each reported recovery timepoint following a period of 6-degree-head-down-tilt bed rest. For each recovery timepoint the effect was categorize as "Returned to baseline" or "Not returned to baseline". Returned to Baseline? = Y: Whenever the mean % change equals 0% or reverts from + to—/– to +; Returned to Baseline? = N: Whenever the mean % change remains + or –.All values are displayed as Mean % Change from Baseline (SD); BMC: bone mineral content; BMD: bone mineral density; Y: yes; N: no.*
[a]*Data extracted from figure using WebPlotDigitizer.*
[b]*Data presented as Mean (SEM); N.A.: not available.*

Tesch, 2004; Linnarsson et al., 2006; Alkner et al., 2016) of the included studies. Two studies (Alkner and Tesch, 2004; Alkner et al., 2016) failed to address whether the head-down tilt was maintained throughout the entire bedrest period, whilst one study (Liu et al., 2015) reported that participants were allowed to use the bathroom for 5–10 min a day.

3.2 Study Characteristics

From the 18 studies, selected data were extracted from 169 participants (11 females, 6.5%) within control/no-countermeasure groups with ages ranging between 20 (Stegemann et al., 1985) and 45 (Convertino et al., 1990) years old. The duration of –6° HDTBR ranged between five (Rittweger et al., 2015) and 120 days (Linnarsson et al., 2006), and included five studies of short duration (5–14 days) HDTBR (Stegemann et al., 1985; Beck et al., 1992; Schulz et al., 1992; Samel et al., 1993; Rittweger et al., 2015), two studies of medium (15–59 days) duration (Convertino et al., 1990; Ferretti et al., 2001), and 11 studies of long duration (≥60 days) HDTBR (Alkner and Tesch, 2004; Linnarsson et al., 2006; Rittweger et al., 2007; Rittweger and Felsenberg, 2009; Belavý et al., 2011a; Beller et al., 2011; Liu et al., 2015; Alkner et al., 2016; Westby et al., 2016; Belavý et al., 2017;

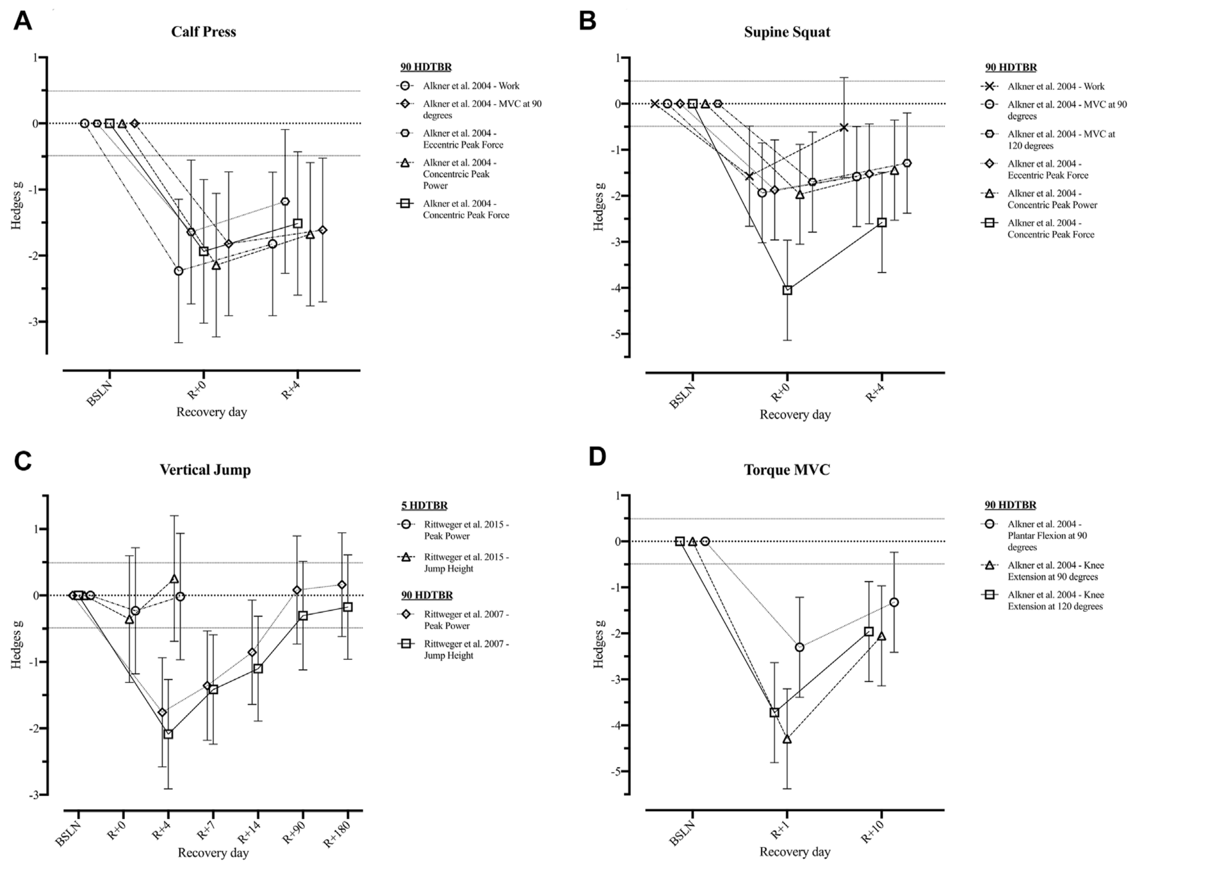

FIGURE 3 | Visualisation of the recovery of outcomes related to the '*Muscular System*' after a period of head-down-tilt bed rest, displayed as Hedges *g* with 95% Confidence Interval. **(A)** Recovery of outcomes related to the performance of a Calf Press **(B)** Recovery of outcomes related to the performance of a Supine Squat **(C)** Recovery of outcomes related to the performance of a Vertical Jump **(D)** Recovery of the Torque generated during performance of a Maximal Voluntary Contraction of the lower limb. To determine whether a particular outcome could be deemed as "recovered" during the recorded recovery period, the Westlake's Confidence Interval Procedure (Seaman and Serlin, 1998) was used. This procedure tests for equivalence between two means using a confidence interval. To do so, Upper (0.49) and Lower (−0.49) equivalency bounds of interest were determined, corresponding to the limit of a small effect size. When combined with the 95% Confidence Interval of the Hedges *g*, three scenarios are possible: 1) *No evidence of recovery*: It cannot be concluded that the difference between means is trivial as the 95% Confidence Interval falls completely outside the set equivalency bounds; 2) *Weak evidence of recovery*: The results are inconclusive as the 95% Confidence Interval partially falls within the set equivalency bounds, thus including both trivial and non-trivial differences. The dotted lines at 0.49 and −0.49 represent the upper and lower equivalency bounds; 3) *Strong evidence of recovery:* There is practical equivalence as the 95% Confidence Interval falls completely within set equivalency bounds; 5 HDTBR: Included studies implementing a head-down-tilt bed rest period of 5 days (Rittweger et al., 2015); 90 HDTBR: Included studies implementing a head-down-tilt bed rest period of 90 days (Alkner and Tesch, 2004; Rittweger et al., 2007); R+. . .: Respective recovery day; MVC: Maximal Voluntary Contraction.

Kramer et al., 2017), as categorized by Sundblad and Orlov (2014). Reported recovery periods lasted between 2 (Samel et al., 1993), and 360 days (Rittweger and Felsenberg, 2009; Belavý et al., 2017) (**Table 2**).

3.3 Recovery of 'Anthropometrics Outcomes'

None of the short and medium duration HDTBR studies reported on the recovery of anthropometric outcomes. Two long duration HDTBR studies (Rittweger et al., 2007; Westby et al., 2016) provided sufficient data to calculate effect sizes for outcomes related to anthropometric outcomes (**Figure 2** and ***Supplementary Table S2***). Following 60 days of HDTBR, mean body weight and BMI returned to baseline values by R+3, with Hedges *g* of 0.00 [−1.31; 1.31] (Westby et al., 2016). Following a 90-days HDTBR, body weight increased during the recovery period and surpassed the baseline value by R+90 (g = 0.10 [−0.71; 0.92]) (Rittweger et al., 2007).

One long duration HDTBR study (Kramer et al., 2017) reported the percentage change from baseline for total body mass, fat mass and lean mass (**Table 3**), indicating an increase in total body mass and lean mass from R+7 to R+14, while fat mass decreased. Yet, none of the outcomes returned to baseline within the recorded recovery period.

3.4 Recovery of the 'Skeletal System'

None of the included studies reported sufficient information to calculate effect sizes of operationally relevant outcomes related to the skeletal system.

TABLE 5 | % Change with SD of outcome parameters related to the *'Muscular System'*.

Recovery timepoint	Author			Returned to baseline? = Y
	Alkner et al. (2016)	Belavý et al. (2017)	Ferretti et al. (2001)	
	90 days HDTBR	***90 days HDTBR***	***42 days HDTBR***	
Muscle Volume—Lateral Gastrocnemius				
R+13		–11.8 (11.6)		
Returned to Baseline? (Y/N)		N		0/1
R+90		2.6 (9.6)		
Returned to Baseline? (Y/N)		Y		1/1
R+180		1.1 (10.1)		
Returned to Baseline? (Y/N)		Y		1/1
R+360		3.0 (10.5)		
Returned to Baseline? (Y/N)		Y		1/1
Muscle Volume—Medial Gastrocnemius				
R+13		–7.6 (8.4)		
Returned to Baseline? (Y/N)		N		0/1
R+90		2.7 (6.4)		
Returned to Baseline? (Y/N)		Y		1/1
R+180		2.5 (4.8)		
Returned to Baseline? (Y/N)		Y		1/1
R+360		3.9 (6.0)		
Returned to Baseline? (Y/N)		Y		1/1
Muscle Volume—Soleus				
R+13		–5.5 (6.1)		
Returned to Baseline? (Y/N)		N		0/1
R+90		2.4 (4.6)		
Returned to Baseline? (Y/N)		Y		1/1
R+180		3.9 (3.2)		
Returned to Baseline? (Y/N)		Y		1/1
R+360		4.3 (5.0)		
Returned to Baseline? (Y/N)		Y		1/1
Muscle Volume—Vasti				
R+13		–10.1 (6.7)		
Returned to Baseline? (Y/N)		N		0/1
R+90		0.9 (7.5)		
Returned to Baseline? (Y/N)		Y		1/1
R+180		1.5 (6.8)		
Returned to Baseline? (Y/N)		Y		1/1
R+360		3.1 (8.5)		
Returned to Baseline? (Y/N)		Y		1/1
Muscle Volume—Rectus Femoris				
R+13		–4.2 (6.2)		
Returned to Baseline? (Y/N)		N		0/1
R+90		–0.8 (6.0)		
Returned to Baseline? (Y/N)		N		0/1
R+180		–0.1 (5.4)		
Returned to Baseline? (Y/N)		N		0/1
R+360		1.0 (6.9)		
Returned to Baseline? (Y/N)		Y		1/1
Muscle Volume—Biceps Femoris Long Head				
R+13		–10.5 (7)		
Returned to Baseline? (Y/N)		N		0/1
R+90		2.7 (6.9)		
Returned to Baseline? (Y/N)		Y		1/1
R+180		1.7 (4.9)		
Returned to Baseline? (Y/N)		Y		1/1
R+360		2.2 (6)		
Returned to Baseline? (Y/N)		Y		1/1

(Continued on following page)

TABLE 5 | (*Continued*) % Change with SD of outcome parameters related to the *'Muscular System'*.

Recovery timepoint	Author			Returned to baseline? = Y
	Alkner et al. (2016)	Belavý et al. (2017)	Ferretti et al. (2001)	
	90 days HDTBR	***90 days HDTBR***	***42 days HDTBR***	
Muscle Volume—Biceps Femoris Short Head				
R+13		–0.2 (7.1)		
Returned to Baseline? (Y/N)		N		0/1
R+90		4.5 (8.5)		
Returned to Baseline? (Y/N)		Y		1/1
R+180		3 (7.8)		
Returned to Baseline? (Y/N)		Y		1/1
R+360		3.1 (8.4)		
Returned to Baseline? (Y/N)		Y		1/1
Muscle Volume—Semimembranosus				
R+13		–8.4 (6.5)		
Returned to Baseline? (Y/N)		N		0/1
R+90		2.4 (4.5)		
Returned to Baseline? (Y/N)		Y		1/1
R+180		2.7 (3.7)		
Returned to Baseline? (Y/N)		Y		1/1
R+360		4.3 (6)		
Returned to Baseline? (Y/N)		Y		1/1
Muscle Volume—Semitendinosus				
R+13		–3.6 (6)		
Returned to Baseline? (Y/N)		N		0/1
R+90		2.2 (5.8)		
Returned to Baseline? (Y/N)		Y		1/1
R+180		2.2 (6.4)		
Returned to Baseline? (Y/N)		Y		1/1
R+360		2 (7.6)		
Returned to Baseline? (Y/N)		Y		1/1
Muscle Volume—Popliteus				
R+13		-0.4 (8.2)		
Returned to Baseline? (Y/N)		N		0/1
R+90		2.4 (7.4)		
Returned to Baseline? (Y/N)		Y		1/1
R+180		–0.7 (6.2)		
Returned to Baseline? (Y/N)		N		0/1
R+360		1.8 (8.3)		
Returned to Baseline? (Y/N)		Y		1/1
Muscle Volume—Lower Gluteus Maximus				
R+13		–4.5 (6.4)		
Returned to Baseline? (Y/N)		N		0/1
R+90		1.6 (7.2)		
Returned to Baseline? (Y/N)		Y		1/1
R+180		0.8 (8.1)		
Returned to Baseline? (Y/N)		Y		1/1
R+360		5.5 (10.9)		
Returned to Baseline? (Y/N)		Y		1/1
Muscle Volume—Iliopsoas				
R+13		0.5 (9.5)		
Returned to Baseline? (Y/N)		Y		1/1
R+90		–1.3 (9)		
Returned to Baseline? (Y/N)		N		0/1
R+180		0.4 (10)		
Returned to Baseline? (Y/N)		Y		1/1
R+360		1.7 (9.7)		
Returned to Baseline? (Y/N)		Y		1/1

(Continued on following page)

TABLE 5 | (*Continued*) % Change with SD of outcome parameters related to the *'Muscular System'*.

Recovery timepoint	Author			Returned to baseline? = Y
	Alkner et al. (2016)	Belavý et al. (2017)	Ferretti et al. (2001)	
	90 days HDTBR	***90 days HDTBR***	***42 days HDTBR***	
Maximal Voluntary Contraction—Quadriceps				
R+0	−45 (n.a.)			
Returned to Baseline? (Y/N)	N			0/1
R+4	−36 (n.a.)			
Returned to Baseline? (Y/N)	N			0/1
Maximal Absolute Muscle Power during Vertical Jump				
R+2			−23.7 (6.9)	
Returned to Baseline? (Y/N)			N	0/1
R+6			−20.9 (3.4)	
Returned to Baseline? (Y/N)			N	0/1
R+48			−3.8 (n.a.)	
Returned to Baseline? (Y/N)			N	0/1
Maximal Muscle Power normalized to body weight during Vertical Jump				
R+2			−22.7 (5.4)	
Returned to Baseline? (Y/N)			N	0/1
R+6			−20.2 (1.6)	
Returned to Baseline? (Y/N)			N	0/1
R+48			−4.7 (n.a.)	
Returned to Baseline? (Y/N)			N	0/1
Maximal Contraction Force from Vertical Jump				
R+2			−14.7 (5.5)	
Returned to Baseline? (Y/N)			N	0/1
R+6			−11.8 (5.2)	
Returned to Baseline? (Y/N)			N	0/1

Notes. *data of outcome parameters related to the muscular system, reported as % change from baseline were extracted and displayed without any alterations for each reported recovery timepoint following a period of 6-degree-head-down-tilt bed rest. For each recovery timepoint the effect was categorize as "Returned to baseline" or "Not returned to baseline". Returned to Baseline? = Y: Whenever the mean % change equals 0% or reverts from + to—/– to +; Returned to Baseline? = N: Whenever the mean % change remains + or –.All values are displayed as Mean % Change from Baseline (SD); Y: yes; N: no.Data extracted from figure using WebPlotDigitizer. N.A.: not available.*

Three long duration HDTBR studies (Rittweger and Felsenberg, 2009; Belavý et al., 2011a; Beller et al., 2011) provided information on the percentage change from baseline for outcomes related to bone mineral content (BMC) and bone mineral density (BMD) (**Table 4**). Following a 60-days HDTBR (Belavý et al., 2011a), lumbar spine BMC returned to baseline values by R+14, while total body BMC, legs BMC and distal tibia BMC increased, and trunk BMC decreased between R+3 and R+90, but did not return to baseline. For BMD of hip and distal tibia, Beller et al. (2011) reported an increase in BMD, yet values did not reach baseline values at R+360 following a 60-days HDTBR period. Lumbar spine BMD did however show an increase—compared to baseline—in the period of R+4 to R+180 yet was decreased at R+360. Rittweger and Felsenberg (2009) reported, after a 90-days HDTBR period, an increase of both the proximal (4%) and distal (66%) tibia BMC between R+4 and R+360, while only the distal tibia BMC surpassed the baseline values by R+360.

3.5 Recovery of the 'Muscular System'

Three of the included studies (Rittweger et al., 2007, 2015; Alkner et al., 2016) reported a total of 16 operationally relevant outcomes related to the muscular system of which Hedges g effect sizes could be calculated (**Figure 3** and ***Supplementary Table S3***). Following a short HDTBR study of 5 days (Rittweger et al., 2015), jump height returned to baseline at R+4 (g = 0.25 [−0.69; 1.20]), while peak power during vertical jumping did not (g = −0.02 [−0.97; 0.93]). For the same parameters following a long duration HDTBR of 90 days (Rittweger et al., 2007), jump height did not return to baseline within the recorded recovery period of 180 days, while peak power did recover by R+90 (g = 0.08 [−0.73; 0.90]). Outcomes reported by Alkner et al. (2016) following a 90-days bed rest period did show improvement during the 10 days recorded recovery period, but did not fully recover.

One long duration HDTBR study (Belavý et al., 2017) reported the % change of lower limb muscle volumes which returned to baseline values by R+90, except for the recuts femoris muscle volume which only returned to baseline by R+360, following a 42-days bed rest period (**Table 5**). One medium (42 days; (Ferretti et al., 2001)) and one long (90 days; (Alkner et al., 2016)) duration study, although showing improvements in outcomes, did not report any of the outcomes to return to baseline within 4 (Alkner et al., 2016) to 48 days of recovery (Ferretti et al., 2001) (**Table 5**).

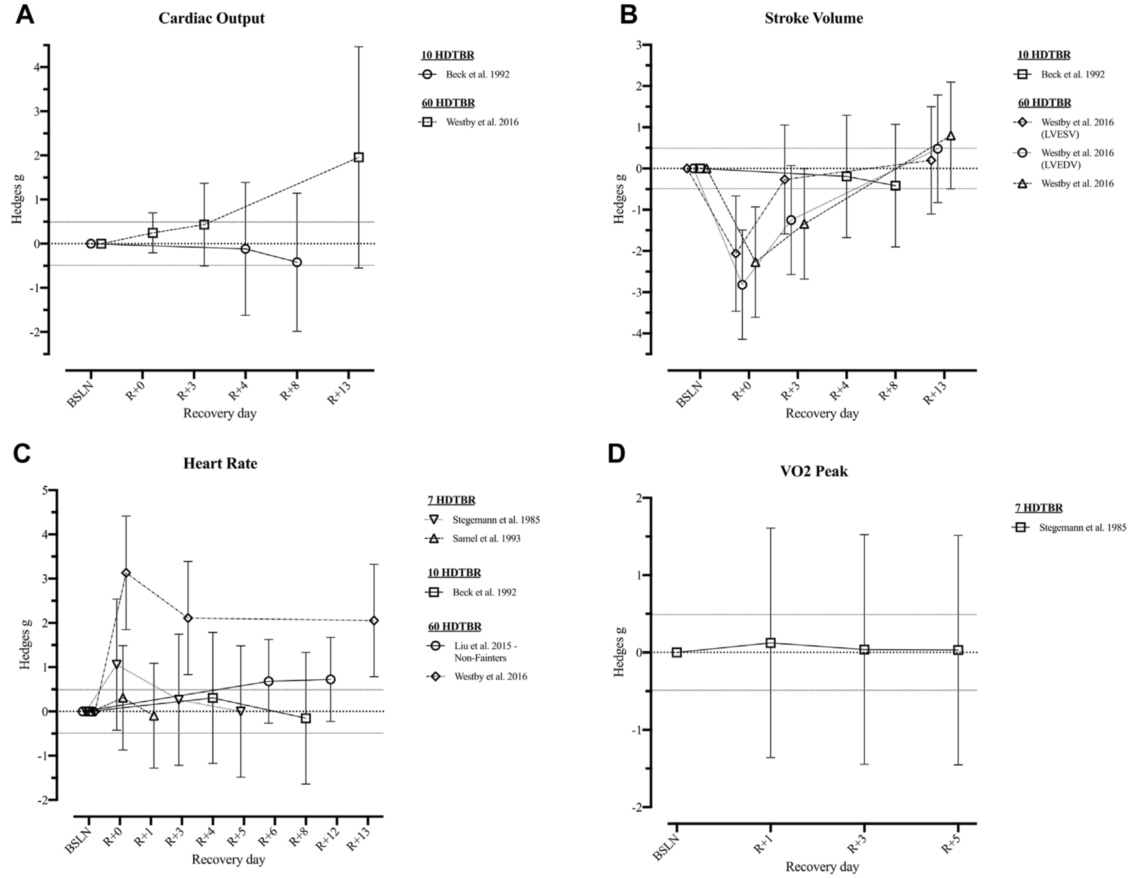

FIGURE 4 | Visualisation of the recovery of outcomes related to the '*Cardiovascular System*' (Part 1) after a period of head-down-tilt bed rest, displayed as Hedges *g* with 95% Confidence Interval. **(A)** Recovery of the Cardiac Output at rest **(B)** Recovery of outcomes related to the Stroke Volume at rest **(C)** Recovery of the resting Heart Rate **(D)** Recovery of the VO2 peak. To determine whether a particular outcome could be deemed as "recovered" during the recorded recovery period, the Westlake's Confidence Interval Procedure (Seaman and Serlin, 1998) was used. This procedure tests for equivalence between two means using a confidence interval. To do so, Upper (0.49) and Lower (−0.49) equivalency bounds of interest were determined, corresponding to the limit of a small effect size. When combined with the 95% Confidence Interval of the Hedges *g*, three scenarios are possible: 1) *No evidence of recovery*: It cannot be concluded that the difference between means is trivial as the 95% Confidence Interval falls completely outside the set equivalency bounds; 2) *Weak evidence of recovery*: The results are inconclusive as the 95% Confidence Interval partially falls within the set equivalency bounds, thus including both trivial and non-trivial differences. The dotted lines at 0.49 and −0.49 represent the upper and lower equivalency bounds; 3) *Strong evidence of recovery:* There is practical equivalence as the 95% Confidence Interval falls completely within set equivalency bounds; 7 HDTBR: Included studies implementing a head-down-tilt bed rest period of 7 days (Stegemann et al., 1985; Samel et al., 1993); 10 HDTBR: Included studies implementing a head-down-tilt bed rest period of 10 days (Beck et al., 1992); 60 HDTBR: Included studies implementing a head-down-tilt bed rest period of 60 days (Liu et al., 2015; Westby et al., 2016); R+…: Respective recovery day; LVESV: Left Ventricular End Systolic Volume; LVEDV: Left Ventricular End Diastolic Volume.

3.6 Recovery of the 'Cardiovascular System'

Following short duration HDTBR, cardiac output, stroke volume, systolic and diastolic blood pressure, and mean arterial pressure remained decreased at R+8 following 10 days bed rest (Beck et al., 1992) Resting heart rate on the other hand returned to baseline between R+1 (Samel et al. (1993); 7 days HDTBR) and R+8 (Beck et al. (1992); 10 days HDTBR). Following a 7-days HDTBR (Stegemann et al., 1985), VO2 peak measured while using a bicycle ergometer revealed an initial increase at R+1 (g = 0.12 [−1.36; 1.61]) but decreased during the following days (R+5: g = 0.03 [−1.45; 1.51]). Details on recovery following medium duration HDTBR were limited to the diastolic and systolic blood pressure and mean arterial pressure, as reported by Convertino et al. (1990), which elevated throughout the recovery period (R+2—R+30). Recovery of cardiovascular outcomes after long duration (60-days) HDTBR were reported by Westby et al. (2016) and Liu et al. (2015). Results of Westby et al. (2016) indicated cardiac output was elevated on R+0 (g = 0.25 [−0.21; 0.70]) and increased during the following days (R+13: g = 1.96 [−0.55; 4.46]). The same is noted for stroke volume and the left ventricular end systolic/diastolic volume, although at R+0 a reduction is noted (g = −2.27 [−3.61; 0.93]; −2.06 [−3.46; −0.66] and −2.82 [−4.15; −1.49] respectively) baseline values are surpassed at R+13 (g = 0.80 [−0.49; 2.10]; 0.20 [−1.10; 1.50] and 0.48 [−0.83; 1.78] respectively). Results on the recovery of heart rate at rest are contradictory as Westby et al. (2016) reported a decrease during the 13-days recovery period, while Liu et al. (2015) reported an increase during the 12-days recovery period, the same trend could be noted for the recovery of the mean arterial pressure. Hedges g values of the included

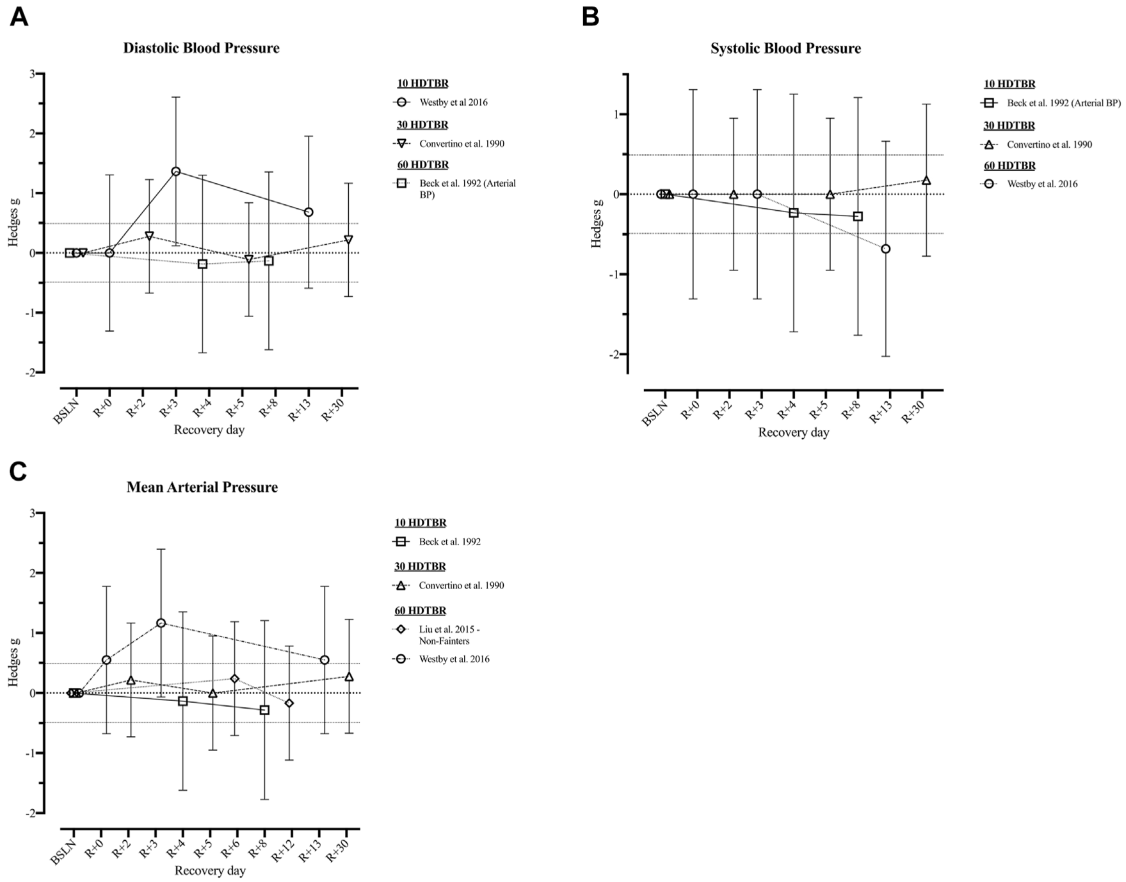

FIGURE 5 | Visualisation of the recovery of outcomes related to the '*Cardiovascular System*' (Part 2) after a period of head-down-tilt bed rest, displayed as Hedges *g* with 95% Confidence Interval. **(A)** Recovery of the Diastolic Blood Pressure **(B)** Recovery of the Systolic Blood Pressure **(C)** Recovery of Mean Arterial Pressure. To determine whether a particular outcome could be deemed as "recovered" during the recorded recovery period, the Westlake's Confidence Interval Procedure (Seaman and Serlin, 1998) was used. This procedure tests for equivalence between two means using a confidence interval. To do so, Upper (0.49) and Lower (–0.49) equivalency bounds of interest were determined, corresponding to the limit of a small effect size. When combined with the 95% Confidence Interval of the Hedges *g*, three scenarios are possible: 1) *No evidence of recovery*: It cannot be concluded that the difference between means is trivial as the 95% Confidence Interval falls completely outside the set equivalency bounds; 2) *Weak evidence of recovery*: The results are inconclusive as the 95% Confidence Interval partially falls within the set equivalency bounds, thus including both trivial and non-trivial differences. The dotted lines at 0.49 and –0.49 represent the upper and lower equivalency bounds; 3) *Strong evidence of recovery:* There is practical equivalence as the 95% Confidence Interval falls completely within set equivalency bounds; 10 HDTBR: Included studies implementing a head-down-tilt bed rest period of 10 days (Westby et al., 2016); 30 HDTBR: Included studies implementing a head-down-tilt bed rest period of 30 days (Convertino et al., 1990); 60 HDTBR: Included studies implementing a head-down-tilt bed rest period of 60 days (Beck et al., 1992; Liu et al., 2015; Westby et al., 2016); R+. . .: Respective recovery day; Arterial BP**:** Arterial systolic and diastolic Blood Pressure.

outcomes are presented in **Figures 4**, **5** (***Supplementary Table S4***).

Reportings on the percentage change from baseline were limited to heart rate, systolic and diastolic blood pressure and mean arterial pressure (**Table 6**). Following a 10-days HDTBR period (Schulz et al., 1992), heart rate showed an increase from R+0 to R+15, remained stable during the first 14 days post 60-days HDTBR (Kramer et al., 2017), and did not change within the initial 3 days of recovery following 120 days of HDTBR (Linnarsson et al., 2006). For systolic blood pressures, a reduction was noted from R+1 to R+14 after 60 days HDTBR (Kramer et al., 2017), while staying stable but elevated during the initial 14 days of recovery following 120 days of bed rest (Linnarsson et al., 2006), which was also the case for diastolic blood pressure (Kramer et al., 2017). Mean arterial pressure showed a slight decrease during the initial 4 days of recovery following 10 days of HDTBR (Schulz et al., 1992), while remaining elevated up to R+14 after 120 days of bed rest (Linnarsson et al., 2006).

4 DISCUSSION

As the passive control groups of HDTBR well represented the concept of exercise holiday, we set out to explore the recovery dynamics of this group rather than elaborating on 'adequate' or 'inadequate' exercise-countermeasures of the intervention group. By doing so, the potential advantages of exercise holidays in accordance with the needs of and shifts in future crewed space

TABLE 6 | % Change with SD of outcome parameters related to the *'Cardiovascular System'*.

Recovery timepoint	Author			Returned to baseline? = Y
	Kramer et al. (2017)	Linnarsson et al. (2006)	Schulz et al. (1992)	
	60 days HDTBR	***120 days HDTBR***	***10 days HDTBR***	
Heart Rate				
R+0	13.05 (13.05) [a]	12.73 (n.a)[a]	2 (9)	
Returned to Baseline? (Y/N)	N	N	N	0/3
R+1	32.47 (14.98) [a]			
Returned to Baseline? (Y/N)	N			0/1
R+2	24.18 (15.65) [a]			
Returned to Baseline? (Y/N)	N			0/1
R+3	22.15 (13.17) [a]	12.73 (n.a.)[a]		
Returned to Baseline? (Y/N)	N	N		0/2
R+4	14.26 (12.32) [a]		-1 (10)	
Returned to Baseline? (Y/N)	N		Y	1/2
R+5	12.43 (8.56) [a]			
Returned to Baseline? (Y/N)	N			0/1
R+6	8.85 (9.93) [a]			
Returned to Baseline? (Y/N)	N			0/1
R+7	10.71 (7.27) [a]			
Returned to Baseline? (Y/N)	N			0/1
R+8	13.86 (10.51) [a]			
Returned to Baseline? (Y/N)	N			0/1
R+9	6.68 (8.56) [a]			
Returned to Baseline? (Y/N)	N			0/1
R+10	13.01 (12.50) [a]			
Returned to Baseline? (Y/N)	N			0/1
R+11	8.32 (8.99) [a]			
Returned to Baseline? (Y/N)	N			0/1
R+12	10.13 (15.96) [a]			
Returned to Baseline? (Y/N)	N			0/1
R+13	11.24 (10.68) [a]			
Returned to Baseline? (Y/N)	N			0/1
R+14	12.04 (8.56) [a]			
Returned to Baseline? (Y/N)	N			0/1
R+15		9.17 (n.a)[a]		
Returned to Baseline? (Y/N)		N		0/1
Systolic Blood Pressure				
R+0	1.65 (3.15) [a]	10.94 (n.a.) [a]		
Returned to Baseline? (Y/N)	N	N		0/1
R+1	−2.31 (5.39) [a]			
Returned to Baseline? (Y/N)	Y			1/1
R+2	−4.24 (5.45) [a]			
Returned to Baseline? (Y/N)	Y			1/1
R+3	−3.16 (6.81) [a]	10.41 (n.a.) [a]		
Returned to Baseline? (Y/N)	Y	N		1/2
R+4	−2.88 (6.20) [a]			
Returned to Baseline? (Y/N)	Y			1/1
R+5	−2.65 (6.77) [a]			
Returned to Baseline? (Y/N)	Y			1/1
R+6	−5.21 (7.38) [a]			
Returned to Baseline? (Y/N)	Y			1/1
R+7	−8.27 (5.61) [a]			
Returned to Baseline? (Y/N)	Y			1/1
R+8	−4.30 (4.39) [a]			
Returned to Baseline? (Y/N)	Y			1/1
R+9	−8.28 (4.06) [a]			
Returned to Baseline? (Y/N)	Y			1/1
R+10	−7.23 (3.45) [a]			
Returned to Baseline? (Y/N)	Y			1/1
R+11	−5.87 (5.00) [a]			
Returned to Baseline? (Y/N)	Y			1/1
R+12	−5.03 (7.40) [a]			
Returned to Baseline? (Y/N)	Y			1/1

(Continued on following page)

TABLE 6 | (*Continued*) % Change with SD of outcome parameters related to the *'Cardiovascular System'*.

Recovery timepoint	Author			Returned to baseline? = Y
	Kramer et al. (2017)	Linnarsson et al. (2006)	Schulz et al. (1992)	
	60 days HDTBR	*120 days HDTBR*	*10 days HDTBR*	
R+13	−5.05 (4.23) [a]			
Returned to Baseline? (Y/N)	Y			1/1
R+14	−4.56 (6.24) [a]	10.32 (n.a.) [a]		
Returned to Baseline? (Y/N)	Y	N		1/2
Diastolic Blood Pressure				
R+0	6.48 (4.32) [a]			
Returned to Baseline? (Y/N)	N			0/1
R+1	2.74 (6.58) [a]			
Returned to Baseline? (Y/N)	N			0/1
R+2	−3.94 (6.58) [a]			
Returned to Baseline? (Y/N)	Y			1/1
R+3	−4.45 (12.03) [a]			
Returned to Baseline? (Y/N)	Y			1/1
R+4	−5.42 (9.16) [a]			
Returned to Baseline? (Y/N)	Y			1/1
R+5	−2.90 (7.90) [a]			
Returned to Baseline? (Y/N)	Y			1/1
R+6	−7.35 (11.16) [a]			
Returned to Baseline? (Y/N)	Y			1/1
R+7	−11.61 (8.90) [a]			
Returned to Baseline? (Y/N)	Y			1/1
R+8	−9.55 (6.90) [a]			
Returned to Baseline? (Y/N)	Y			1/1
R+9	−12.42 (4.84) [a]			
Returned to Baseline? (Y/N)	Y			1/1
R+10	−9.32 (4.84) [a]			
Returned to Baseline? (Y/N)	Y			1/1
R+11	−7.07 (5.84) [a]			
Returned to Baseline? (Y/N)	Y			1/1
R+12	−5.61 (9.55) [a]			
Returned to Baseline? (Y/N)	Y			1/1
R+13	−7 (6.29) [a]			
Returned to Baseline? (Y/N)	Y			1/1
R+14	−4.65 (6.77) [a]			
Returned to Baseline? (Y/N)	Y			1/1
Mean Arterial Pressure				
R+0		4.92 (n.a.) [a]	-6 (5)	
Returned to Baseline? (Y/N)		N	N	0/2
R+3		4.52 (n.a.) [a]		
Returned to Baseline? (Y/N)		N		0/1
R+4			-4 (5)	
Returned to Baseline? (Y/N)			N	0/1
R+15		4.92 (n.a.) [a]		
Returned to Baseline? (Y/N)		N		0/1

Notes. *data of outcome parameters related to the cardiovascular system, reported as % change from baseline were extracted and displayed without any alterations for each reported recovery timepoint following a period of 6-degree-head-down-tilt bed rest. For each recovery timepoint the effect was categorize as "Returned to baseline" or "Not returned to baseline". Returned to Baseline? = Y: Whenever the mean % change equals 0% or reverts from + to—/– to +; Returned to Baseline? = N: Whenever the mean % change remains + or –.All values are displayed as Mean % Change from Baseline (SD); Y: yes; N: no.*
[a]*Data extracted from figure using WebPlotDigitizer.*

missions could be highlighted, to be included and explored upon in future evidence-based countermeasure programmes. Main findings on the post-HDTBR recovery dynamics of the passive control group include: 1) anthropometric outcomes show steady improvements, with a possible return to baseline between R+3 and R+90; 2) recovery of BMC and BMD of the lower limbs reveals a continued decrease up to R+14, followed by steady improvements but failing to fully recover by R+360; 3) lower limb muscle volumes show a consistent recovery by R+90; and 4) independent of HDBTR campaign duration, cardiovascular outcomes showed trends of normalization within the initial 14 days of recovery.

As future LEO and exploration missions will differ in duration, for the sake of clarity, the current literature will primarily be discussed considering the upcoming Artemis and Lunar Gateway missions that are slated to take between 30 and 90 days (Gerstenmaier and Crusan, 2018; NASA, 2021). Such mission durations are consistent with the HDTBR durations used in almost all of the included literature: 30 days (Convertino et al., 1990), 42 days (Ferretti et al., 2001), 60 days (Belavý et al., 2011a; Beller et al., 2011; Liu et al., 2015; Westby et al., 2016; Kramer et al., 2017), and 90 days (Alkner and Tesch, 2004; Rittweger et al., 2007; Rittweger and Felsenberg, 2009; Alkner et al., 2016; Belavý et al., 2017). The remaining six HDTBR studies were of shorter duration: 5 days (Rittweger et al., 2015), 7 days (Stegemann et al., 1985; Samel et al., 1993), 10 days (Beck et al., 1992; Schulz et al., 1992), except one which was 120 days (Linnarsson et al., 2006).

4.1 Anthropometric Outcomes

In space, loss of body mass appears to be highly variable, but the average rate has been estimated to be around 2.4% per 100 days spent in space (Matsumoto et al., 2011). However, this must be contextualised by the fact that astronauts are all performing extensive countermeasures (Petersen et al., 2016). In contrast, current HDTBR data without exercise countermeasures demonstrates reduced body mass, during and shortly after long duration HDTBR (Hedges g = [−0.43; −0.25], **Figure 2**, (Rittweger et al., 2007; Westby et al., 2016; Kramer et al., 2017)). Body mass decrements may be precipitated by changes in blood volume (Tavassoli, 1982; Kunz et al., 2017), muscle atrophy (LeBlanc et al., 2000; Alkner and Tesch, 2004; Winnard et al., 2019) and/or bone demineralization (LeBlanc et al., 2000; Rittweger et al., 2005; Belavý et al., 2011b). A potentially important driver for body mass loss may be negative energy balance due to the mismatch between energy intake and energy expenditure (Stein, 2000; Laurens et al., 2019). Data presented by Stein (2000) suggests a moderate positive relationship between the total energy expenditure and loss of body mass during spaceflight. Thus, the increased energy expenditure associated with exercise countermeasures appears not to be accompanied by increased energy intake, resulting in a negative energy balance. In fact, a negative nitrogen balance—suggesting loss of muscle mass—was also reported in-flight—despite performing exercise countermeasures (Stein, 2000; Stein, 2013). However, interestingly during the first 2 weeks of the Space Life Sciences (SLS) 1 and 2 Shuttle missions (Stein et al., 1996) where no exercise countermeasures were performed, energy and nitrogen balance were stable, suggesting a muscle mass preservation (Stein, 2000).

In contrast, following 90-days HDTBR, Rittweger et al. (2007) reported that body mass loss was still apparent after 14 days of recovery (Hedges g = −0.25). Similarly, following 60-days HDTBR, Kramer et al. (2017) reported approximately 5% body mass reductions, mostly attributed to lean body mass loss which also did not recover within 14 days post-HDTBR. This body mass loss disparity may be due to the energy intake reported by Kramer et al. (2017) being calculated based on the resting metabolic rate, instead of the actual 24-h energy expenditure (Piaggi et al., 2015; Laurens et al., 2019). In contrast, Westby et al. (2016) adjusted daily caloric intake so that body mass was maintained within 3% of that on the third day of HDTBR. This resulted in the body mass returning to baseline after the third day of recovery (Hedges g = 0.00, **Figure 2**). Thus, depending on HDTBR duration and dietary intake, body mass recovery may occur from 3 days (Westby et al., 2016) up to 3 months, or longer (Rittweger et al., 2007). Yet, based on data of 246 different astronauts over 514 mission, 62% failed to regain all of the lost body mass at a time-interval of R+[91–396] days postflight (Matsumoto et al., 2011), unfortunately, relative changes in lean and adipose body mass are unknown. Sustained reductions in body mass could however contribute to a significant risk of an adverse effects such as reduced stamina or increased risk of muscle injuries (Matsumoto et al., 2011). Especially if loss of lean body mass is evident and results in an operationally meaningful loss of muscle strength (i.e., considerable and in a specific muscle group), thus possibly leading to a crewmember not being able to perform an operational task that they previously could.

4.2 The Skeletal System

In space, the average rate of bone loss has been estimated to be between 0.5% (Stavnichuk et al., 2020) and 1.5% (Lang et al., 2004) per month in the lower limbs, despite in-flight exercise countermeasures (Smith et al., 2012). Thus, the rate of bone loss would presumably be even greater if no countermeasures were being performed. During 60-days HDTBR, Beller et al. (2011) reported tibial bone mineral density loss ranging between 1.1 and 2.0% per month, while for the hip BMD decreased by between 1.5 and 2.0% per month, potentially increasing the risk of fractures. Although BMD loss appears to be slightly greater during HDTBR—with no exercise countermeasures—fortunately it remains far below that observed in spinal cord injury patients. In this cohort, a rapid linear decline of lower extremity BMD results in a loss of ~27% in the first three to 4 months after injury and reaching a plateau at ~37% after 16 months (Biering-Sorensen et al., 1990; Garland et al., 1992), thus substantially increasing the risk of bone fractures (Gernand, 2004).

However, the limited data presented in the current study suggests that bone recovery is slow, potentially taking up to 3 to 4 times that of the unloading period (Gernand, 2004; Orwoll et al., 2013; Stavnichuk et al., 2020). In fact, long duration HDTBR data on lower limb bone mineral content (Rittweger and Felsenberg, 2009; Belavý et al., 2011a) and density (Beller et al., 2011) suggests that the loss continues up to a period of 14 days after HDTBR is concluded (**Table 4**) due to the inertia in bone remodelling regulation. Similarly, bone accrual appears to be evident only after approximately 1 week of reconditioning (Armbrecht et al., 2010). Furthermore, some residual BMD loss appears to persist, which may increase long-term fracture risk. Decrements of BMD of the tibia (−1.81 ± 0.82%) and the hip (−0.52 ± 0.65%) were still present at R+360 after a 60-days HDTBR (Beller et al., 2011). Similarly, loss of BMD postflight was still present 6 months after long-duration spaceflight (Vico et al., 2000), and was even persistent after 5 years in nine Skylab crew members (Tilton et al., 1980). Moreover, Sibonga et al. (2007) determined the ‘50% recovery time’ based on data of 46 long-duration crew members assigned to Mir or ISS missions. This 50% recovery time represents the number of days after landing, needed to restore half of the lost BMD and ranged between 97 days for the Pelvis and 255 days for the Trochanter. Whilst small increases in bone fracture risk may be acceptable when returning to Earth, this could be critical when landing and performing extravehicular activities (EVAs) on the Lunar surface in the absence of medical support (Horneck et al., 2003). Therefore, limits of acceptable losses of BMD—within a spaceflight context—should be defined, as for example has been done for osteopenia (BMD T-score: −2.5 < T-score < −1.0) or osteoporosis (BMD T-score < −2.5) (Woolf and Pfleger, 2003). Determining how close a person gets to a significant increase in risk of low trauma fractures during a period of exercise-free bed rest—and the recovery thereof—is key in determining whether this limit of acceptable bone loss is equal to a pathological threshold (i.e., osteopenia or osteoporosis), or whether an acceptable operational threshold is closer to normal.

4.3 The Muscular System

In space, as with the skeletal system, the muscles most affected are those with a prime 'anti-gravitational' function such as those in the trunk and lower limbs (Stein, 2013; Winnard et al., 2019). Based on the data presented in the review of Winnard et al. (2019) moderate effects (Hedges $g \geq 0.6$) occur within seven to 14 days of HDTBR, while large effects (Hedges $g \geq 1.2$) occur after 28–35 days. Muscle mass is critical to a crewmember's strength and endurance (Gernand, 2004; Winnard et al., 2019). In general, large effects (i.e., reduction) of muscle volume and cross-sectional area were only noted after 28 days of HDTBR, whereas decrements of muscle thickness, maximal torque, and strength after 35 days, whilst large peak power effects were apparent after 56 days (Winnard et al., 2019). Such decrements could impede mission critical tasks such as EVAs or landing operations.

Information on the recovery of maximal voluntary contractions, peak forces, or the work performed during a supine squat or calf press was only reported up until the fourth day of recovery after a 90-day period of HDTBR (Alkner et al., 2016). Although improvements in all related outcomes were noted within 4 days, none returned to baseline (Hedges g = [−2.58; −0.51], **Figure 3**). Based on the information provided by Rittweger et al. (2007) on the peak power generated during jumping after 90-days HDTBR, it could be suggested that muscle outcomes recover within 90 days of recovery. Similarly, data reported by Belavý et al. (2017) after a 90-days HDTBR period indicates that lower limb muscle volume returns to baseline between day 13 and 90 of recovery (**Table 5**). This data concurs with findings of crewmembers returning from a long-duration spaceflight. Restoration of muscle mass and strength of crewmembers during the post-flight rehabilitation period seems to occur at the same rate, or even at a faster rate, of the initial atrophy (Leblanc et al., 1990; Tesch et al., 2005; Petersen et al., 2017). Thus, definition of the imposition of an exercise holiday should consider the high degree of inter-individual variability expressed in muscle outcomes (Gernand, 2004; Stein, 2013; Winnard et al., 2019). Consideration of relative effects should be made as 'stronger' crewmembers may be able to retain operational functionality whilst experiencing greater absolute and relative decrements of their pre-flight muscle mass and strength compared to those with lower pre-flight levels. Definition of 'minimal' strength requirements for spaceflight are critical to inform the implementation of any form of exercise holiday but have yet to be determined (Winnard et al., 2019).

4.4 The Cardiovascular System

Lastly, in space, cardiovascular system outcomes are significantly modulated to adapt to microgravity that negates hydrostatic gradients (Thornton et al., 1987). These changes in blood volume (Beck et al., 1992; Westby et al., 2016; Gallo et al., 2020), cardiac mass (Levine et al., 1997; Westby et al., 2016; Gallo et al., 2020), and aerobic capacity (Levine et al., 1996; Gallo et al., 2020) can be detrimental when returning to Earth or another celestial body. In fact, one of the most common consequences after long duration spaceflight is orthostatic intolerance (Hargens and Richardson, 2009; Liu et al., 2015) which could be critical during landing (Buckey, 2006). Orthostatic intolerance, with associated hypotension and presyncope, usually takes between 3 days (Waters et al., 2002) and 2 weeks (Vasilyeva and Bogomolov, 1991; Cooke et al., 2000) to recover following long-duration spaceflight. However, Fu et al. (2019) reported that contrary to tilt-table testing and still-standing, after 6 months in space, none of the 12 tested astronauts experienced orthostatic intolerance or hypotension during activities of daily living during the initial 24 h on Earth. While after 60-days HDTBR three out of 14 subjects were reported as 'fainters' during a head-up tilt test immediately after bed rest (Liu et al., 2015). Thus, non-exercise countermeasures such as volume resuscitation through water intake (Fu et al., 2019), or repeated exposure to Lower Body Negative Pressure (LBNP) used to mitigate spaceflight-associated neuro-ocular syndrome (SANS) (Harris et al., 2020) may be helpful to reduce cardiovascular deconditioning including orthostatic intolerance (Watenpaugh et al., 2007; Harris et al., 2020) which would support exercise holiday feasibility.

Additionally, some evidence suggests that aerobic capacity decrements are rapid during the first 30 days of spaceflight, after which (with exercise countermeasures) adaptations appear to plateau (Gernand, 2004; Gallo et al., 2020). Aerobic capacity losses may be an issue for EVAs with even moderate reductions potentially limiting a crewmembers' ability to perform Lunar surface operations (Moore et al., 2014). However, with exercise there is increasing evidence to suggest that aerobic capacity recovers, at least in part, in-flight (Gernand, 2004; Moore et al., 2014; Gallo et al., 2020) and with complete recovery within 30 days postflight (Moore et al., 2014). Similarly, a recovery period of 14–30 days is reported in medium duration (20–42 days) HDTBR participants exposed to exercise countermeasures (Convertino et al., 1985; Sundblad et al., 2000). Also following 60-days HDTBR without exercise countermeasures, cardiac mass and function recovered within 14 days (**Table 6**) when participants were subjected to a progressive reconditioning programme (Westby et al., 2016). In contrast, Beck et al. (1992) observed—after a 10-days HDTBR—decrements in cardiac output, stroke volume and blood pressure which remained lower than baseline at recovery day 8 when recovery was not supervised. Yet, reports of the recovery of peak oxygen uptake, a key metric of cardiovascular fitness, is currently lacking as only Stegemann

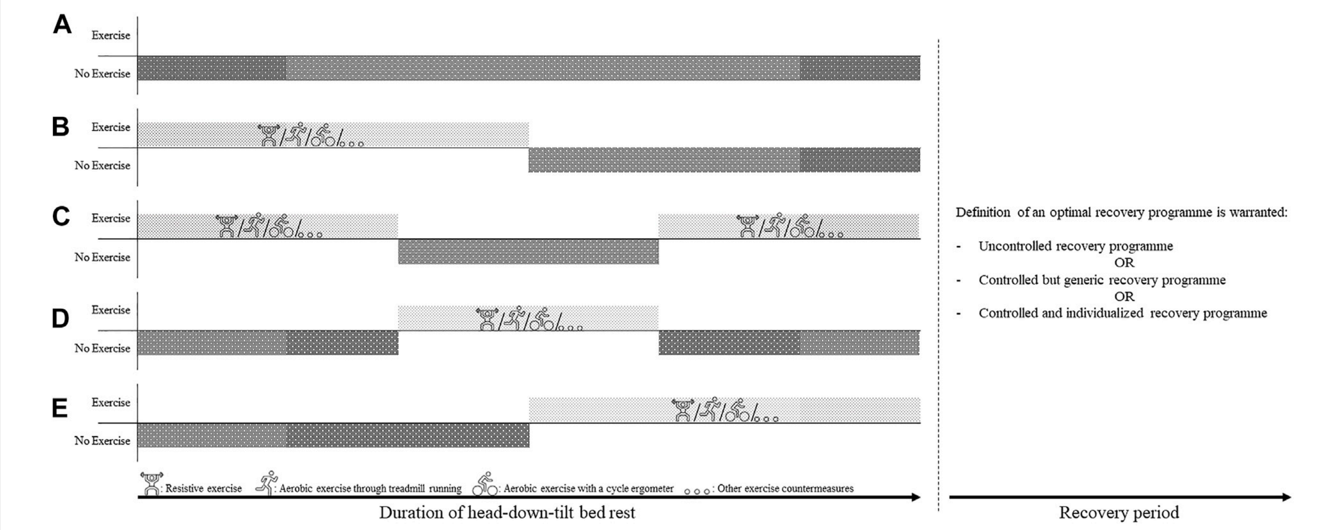

FIGURE 6 | Illustration of possible implementations of Exercise Holidays and possible recovery programme modalities for future head-down-tilt bed rest campaigns. **(A) No Exercise:** The generic 'no exercise' control group wherein participants do not perform any exercise countermeasures through the duration of the bed rest campaign **(B) Exercise First:** Participants start the bed rest campaign with an exercise countermeasure programme, followed by a period of no exercise until the end of the campaign **(C) Exercise—Rest—Exercise:** Participants start and end the bed rest campaign with a period of exercise countermeasures, separated by a period of exercise holidays **(D) Rest—Exercise—Rest:** Participants start and end the bed rest campaign without any exercise (i.e., exercise holiday), separated by a period of exercise countermeasures **(E) Rest First:** Participants start the bed rest campaign with an exercise holiday, followed by a period of performing exercise countermeasures until the end of the campaign. All of the different iterations of bed rest campaigns described above can be combined with different recovery programme modalities during post-bed rest period: Uncontrolled recovery programme: participants are not subjected to a controlled recovery programme. - Controlled but generic recovery programme: all participants complete the same recovery programme, not adapted to the personal needs of the participant. - Controlled and individualized recovery programme: all participants complete an individualize rehabilitation programme, adapted to the personal needs of the participant. Doing so may provide crucial information on the time and resources needed for an optimal recovery to take place.

et al. (1985) reported values after a 7-days HDTBR without exercise, which remained unchanged (Hedges g = [0.03; 0.12]). Based on post-spaceflight data (Perhonen et al., 2001; Trappe et al., 2006), cardiorespiratory responses, heart rate, stroke volume and left ventricular mass are expected to recover during the post-flight rehabilitation phase (Payne et al., 2007).

Importantly, recovery of cardiovascular outcomes could be enhanced when combined with non-exercise countermeasures such as Lower Body Negative Pressure (Harris et al., 2020) and/or fluid volume supplementation (Waters et al., 2005) which are already being implemented in current spaceflight operations (Fu et al., 2019) to minimize the risk of orthostatic intolerance if gravitational loading is to be re-imposed. Additionally, cardiovascular rehabilitation following a protracted period of exercise-free HDTBR may be rapid if the recovery period includes an individualized reconditioning programme (Westby et al., 2016). Future HDTBR studies should therefore aim to investigate the effects of standardized reconditioning programmes during and/or following long-duration HDTBR to increase the evidence base towards implementation of exercise holidays within a spaceflight context.

4.5 Reported Reconditioning Approach After HDTBR Vs Post-spaceflight

In addition to re-exposure to a nominal 1 g loading upon termination of bed rest in HDTBR-participants, and after returning to Earth's gravity in crewmembers, they are subjected to a period of physical reconditioning. Yet, while the reconditioning of crewmembers following spaceflight is well-described (Petersen et al., 2017), most HDTBR studies failed to report any specifics on reconditioning or rehabilitation protocols used during the recovery period. Thirteen out of the 18 included studies only reported the duration of the recovery period without any additional details. One 30-days HDTBR-study reported a 5 day recovery period within the bed rest facility, followed by 25 days (R+6 to R+30) of uncontrolled recovery (Convertino et al., 1990). Kramer et al. (2017) reported that participants were restricted to free movement within the ward during the 15 days recovery period. In Rittweger et al. (2007) and Rittweger and Felsenberg (2009) participants were residing within the facility for 14 days after reambulation during which nutrition was controlled. Only Westby et al. (2016) reported a supervised and progressive reconditioning programme for 10 days, starting at R+4, which included a 1-h supervised ambulation and exercise programme. Throughout the reconditioning period, the intensity, duration, and complexity of the exercises were increased according to the tolerance of the subject with regards to foot tenderness and ankle and knee pain due to the prolonged bed rest. Such an approach demonstrated the recovery of the cardiac mass and function within 2 weeks following a 60-days HDTBR campaign.

The reconditioning approach of Westby et al. (2016) is similar to the highly individualized reconditioning programme used for each ESA crewmember as described by Petersen et al. (2017). In short, each crewmember is supported by a reconditioning team, including an experienced exercise specialist/sport scientist and a physiotherapist. The supervised post-flight reconditioning programme integrates various physiotherapeutic methods and elements from sports and exercise science, resulting in a comprehensive and highly individualized reconditioning programme lasting 21 days. Exercise sessions have a focus on promoting functionality, efficacy, safety, and adequate intensity to optimise neuromusculoskeletal and cardiovascular responses. As large inter-individual variations in postflight condition occur between astronauts, the daily 2-h sessions are adapted to the individual with regards to complexity and intensity. However, the aim for all crewmembers is to be able to perform near, or at the same pre-flight intensity by the end of the 21-days reconditioning programme. Such an intensive post-flight rehabilitation programme is sufficient to make a full recovery of most, but not all aspect of function. Therefore, this is then followed by unsupervised training using an individualised exercise programme aimed at improving, and maintaining, health and fitness over the following months by supporting the neuro-musculoskeletal regeneration process.

This lack of general reporting—or even implementation of—standardized methods or exercise prescriptions during the recovery after HDTBR is an important shortcoming, thus having a profound effect on the ability to compare results across bed rest studies, and to compare the recovery dynamics after prolonged HDTBR with those after actual spaceflight.

4.6 Limitations of the Included Studies

Firstly, although HDTBR is the most robust ground-based analogue to study the effects of prolonged gravitational unloading (Hargens and Vico, 2016), potential confounding factors related to Earth-based analogues need to be taken into account: the inability to completely abolish gravitational stress, and the absence of exposure to space radiation. Although similarities are observed between HDTBR and actual spaceflight, reported changes may appear more rapidly and be more severe during spaceflight as compared to bed rest. Yet, HDTBR is still considered to be a valid analogue despite these limitations (Pavy-Le Traon et al., 2007).

The 18 included studies reported a total of 49 relevant outcome variables across the domains of interest with heterogeneous measurement time points—particularly evident during the recovery periods. This diversity was compounded by a general paucity of data. In addition, inconsistent reporting of mean raw values with standard deviations limited the ability to calculate effect sizes. Thus, effect sizes were only calculated for 27 of the 49 included outcome variables. Even where sufficient information was provided, typically reported sample sizes were low–meaning that caution should be exercised when interpreting this data (Lakens, 2013).

Additionally, vote counting based on the direction of effects was also severely limited due to the inconsistent and heterogenous reporting of outcome measures, with only 4/32 outcome variables reported as percentage change being reported at least twice, thus seriously restricting the generalisability of results. Moreover, differences in baseline reference conditions, especially in cardiovascular outcome variables (e.g., measured while upright/sitting/supine), impairs comparison between studies (Norsk, 2020).

Lastly, significant shortcomings in—the reporting of—the used methodology were indicated by the poor results of quality appraisal of the bed rest methods of included studies (Winnard and Nasser, 2017), thus limiting their comparability.

4.7 Filling in the Gaps—Recommendations for Future Research

It would be desirable if future HDTBR campaigns would implement durations which are directly related to the duration of future Artemis and Lunar Gateway missions, i.e., lasting anywhere between 30 and 90 days, to provide a direct implementation of the gathered knowledge to future exploration missions. Furthermore, these future bed-rest campaigns are also encouraged to explore different implementations of exercise-free periods within the duration of the campaign, as illustrated in **Figure 6**. Additionally, the efficacy of the different exercise devices currently on board the ISS (i.e., ARED, T2 Treadmill, CEVIS), but also the usefulness and efficacy of novel training modalities such as for example plyometric exercises (Weber et al., 2019) should be investigated to define the most optimal exercise regime and get a better understanding of rehabilitation and recovery within a (simulated) microgravity environment. Improving the definition of the optimal in-flight rehabilitation regime—which in its turn could enhance the in-flight recovery of the different physiological systems—could ultimately facilitate the acceptance of any decrements attributed to inactivity during the exercise holiday period, thus potentially increasing the time where crew would not need perform exercise countermeasures, thus enabling them to spend more time on other mission-related tasks, and ultimately to also safe critical resources (Laurens et al., 2019).

In the same way, defining the optimal recovery programme after HDTBR is warranted, as for now recovery after HDTBR is mostly uncontrolled, while crewmembers are provided—both in-flight as well as after returning to Earth—with a comprehensive and individualized exercise programme. Exploring the recovery dynamics of the different physiological systems as a result of either an uncontrolled, a controlled but generic, or a controlled and individualized recovery programme would provide crucial information on the time and resources needed for an optimal recovery to take place.

Moreover, future HDTBR campaigns should also focus on simulating upcoming Lunar Gateway mission profiles where the crew will transition from prolonged exposure to microgravity to hypogravity on the Lunar surface. Consequently, 'conventional' exercise stimuli such as high reaction forces and high muscle forces to stimulate bone growth (Frost, 2003), and high load resistive exercises to promote muscle hypertrophy (Yamada et al., 2012)—as is currently the case—will likely become of secondary importance. For Lunar Gateway missions with Lunar surface EVAs after a prolonged exposure to microgravity, the primary needs and requirements of the countermeasure programmes may probably undergo a shift from focussing on maintaining bone mineral density, muscle strength and VO2max to countermeasures focussing on orthostatic tolerance, postural stability, spatial orientation and balance due to the transition between microgravity and hypogravity, ultimately to assure crew safety and mission success. Such countermeasures mitigating postflight functional and sensorimotor dysfunction were also proposed by Both Miller et al. (2018) and Mulavara et al. (2018) to be incorporated in the in-flight countermeasure portfolio. The current study could be considered as the first step in exploring this potential shift in countermeasure approach as it investigated the recovery of current operationally relevant outcomes after a period of disuse, although it did not take into account the effects of countermeasures targeting postural stability, spatial orientation and balance. Therefore, future research should aim attention at further investigating this shift to aid in defining and evaluating relevant needs and requirements for in-flight countermeasures ensuring crew health and safety in upcoming space exploration missions.

Lastly, within future HDTBR studies, expansion of the current approach for standardized measurements (Sundblad and Orlov, 2014) specifically post-HDTBR is encouraged, including—but not limited to—standardization of post-HDTBR data collection (i.e., daily data collection within the first 14 days of the post-HDTBR period, followed by weekly follow-up data collection up to 3 months or longer) and standardized reporting and publishing of recovery data (i.e., reporting of raw values as means and standard deviations, the use of effect sizes, or a combination of both) thus enabling a more thorough comparison of control groups between studies, and facilitating the feasibility of retrospective analyses.

All the above would add to the body of evidence which would ultimately aid in determining whether the implementation of the concept of exercise holidays within future spaceflight operations—within and beyond LEO—would be feasible and practical.

5 CONCLUSION

The concept of exercise holidays that is presented in the current study should be regarded as one of many steps that are needed to define evidence-based needs and requirements for in-flight exercise countermeasures for future deep space exploration missions. Although a high degree of paucity and inconsistency of reported recovery data is present within the 18 included studies, data suggests that recovery of current operationally relevant outcomes following HDTBR without exercise—and even without targeted exercise rehabilitation during the recovery period—could be timely and does not lead to persistent decrements differing from those experienced following spaceflight. Thus, the concept of exercise holidays looks like a promising concept that should be further explored through space- and ground-based research to fill current knowledge gaps, prior to its potential implementation in human spaceflight exploration missions.

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.

AUTHOR CONTRIBUTIONS

RE, DG, JS, RH, TW and NH were involved in the conception and design of the study; RE, DG, RH, TW and NH were involved in the collection, analysis, and interpretation of the data; RE and NH drafted the manuscript; DG, JS, RH, and TW revised the manuscript; all authors read and approved the manuscript before submission.

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SUPPLEMENTARY MATERIAL

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Effect of Modulated Electromyostimulation on the Motor System of Elderly Neurological Patients. Pilot Study of Russian Currents Also Known as Kotz Currents

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In this brief report, we present preliminary findings from a study of the use of electromyostimulation (EMS) in neurological patients. Assuming the approach to be sufficiently effective, we decided to investigate the motor system of elderly neurological patients before and after a course of Russian currents EMS, which were developed for Soviet athletes and cosmonauts. To this point, 19 patients—EMS (n = 11) and control (n = 8)—have successfully completed the study. The study included patients aged 60–90 years with confirmed walking and balance disorders with a history of chronic cerebral ischemia. Patients in the experimental group underwent a course of modulated EMS of the hip and shin muscles from 3 to 9 procedures. Preliminary results of the study showed good patient acceptance of EMS. After the course, the EMS group showed a significant improvement from baseline in the Tinetti Test (+1.4 points, p = 0.0045), Rivermead Mobility Index (+0.5 points, p = 0.0022), and Timed Up and Go Test (–1.2 s, p = 0.0053). There was also a significant improvement in balance quality of 8.6% (p = 0.04). Shin muscle strength, although trending positively, did not change significantly. There was also no change in hip and shin muscles' tone. No significant changes were observed in the control group in the same tests. It can be concluded that stimulation of the hip and shin muscles with Russian (Kotz) currents has a positive effect on the motor system of elderly neurological patients. Significant effects with a course of short duration indicate that this EMS regimen is promising.

Keywords: electromyostimulation, Russian currents, timed up and go test, neurological patients, postural stability

1 INTRODUCTION

Older age is accompanied by a gradual decline in body functions, including decreased physical activity. Age-related changes are known to affect the motor areas of the brain, and as a consequence, posture, gait and fine motor skills suffer (Seidler et al., 2010), which in turn may mediate an even greater decline in motor activity. Skeletal muscles are particularly susceptible to the effects of aging, and with age they steadily lose function and mass. The decline in functional performance is associated with a general decline in muscle integrity as fibrosis and fat accumulation replace functional contractile tissue, as well as the loss of the fastest and most powerful fibers (Scicchitano et al., 2009; Vinciguerra et al., 2010). Prolonged course of exercises is known to counteract muscle weakness: it increases protein synthesis, metabolism and satellite cell number, stimulates appetite, increases IGF-1 expression levels and capillary bed density (Paffenbarger et al., 1994; Kern et al., 2014). However, it is not always possible to maintain sufficient level of physical activity, and electromyostimulation (EMS) can be an alternative to intensive physical exercise.

The main advantage of the EMS approach to rehabilitation is the wide coverage of patients with a variety of medical histories (Jones et al., 2016). The use of EMS is possible even in cases where physical activity is difficult or impossible due to cardiovascular (Arenja et al., 2021; Poltavskaya et al., 2021), pulmonary (Zanotti et al., 2003) and other conditions (Arija-Blazquez et al., 2014; Nussbaum et al., 2017). Adherence to bed rest has been shown to reduce muscle strength by 5–7% (Pisot et al., 2008). EMS has also been shown not only to increase muscle strength but also to reduce lower limb spasticity after stroke (Moon et al., 2017), and a 1-week course of stimulation of the quadriceps femoris and peroneus longus muscles of both legs results in an increase in hip and shin circumferences (Gerovasili et al., 2009). Thus, EMS is a promising treatment/countermeasure modality (Arenja et al., 2021).

One of the methodological approaches to EMS is the so-called Russian currents developed by Y.M. Kotz's group for Soviet athletes (Kotz and Hvilon, 1971), and it was later adapted for cosmonauts. It is important to note that the method is a unique development that differs from its counterparts (NOT high voltage pulsed current or whole-body EMS, etc.). The essence of this type of stimulation is the modulation of medium frequency current (2.0–5 kHz) by lower frequencies (10–100 Hz). Due to these stimulation parameters, the evoked muscle contractions are as close as possible to physiological ones, which reduce the discomfort of the procedure, patient fatigue, and also has an anesthetic effect in itself (Rampazo and Liebano, 2022). It is also suggested that such a configuration of the electrical signal may help to achieve visible results in a shorter time than with unmodulated currents (Ward and Shkuratova, 2002).

The aim of this work was to assess the motor system using 1) standard clinical questionnaires and tests, measurements of 2) postural stability and 3) muscle tone in elderly patients before and after EMS course with Russian currents. We hypothesised that electromyostimulation using Russian currents, also called Kotz currents, could improve the overall motor system in neurological patients in 10 treatments or less.

2 METHODS

2.1 Participants

To this point, 19 people have successfully completed the study. Two more people dropped out of the study: one for the medical reasons described below, the other for failing to attend the final examination. The patients were initially divided into two subgroups: EMS (n = 11) and control (n = 8). Although the patients of both sexes were considered as subjects in this examination, for one reason or another only woman participated in both subgroups so far. Details of the study participants, including height, weight and cognitive scores on the MMSE scale, are presented in **Supplementary Table S1** of the supplementary materials.

Patients were admitted to the hospital with complaints of dizziness and unsteadiness when walking. After an initial examination by a neurologist, meeting the study criteria (see below) and signing voluntary informed consent, patients were randomly allocated into either the EMC or control groups. Participation in the study did not exclude the performance of physiotherapeutic procedures (magnetotherapy, darsonvalization, electrosleep therapy) and therapeutic exercises (vascular gymnastics and exercises for spinal osteochondrosis). Given the inpatient profile, most patients received standard vascular and metabolic therapy, as well as antidepressants and neuroleptics. The groups were homogeneous in terms of the treatment used.

Inclusion criteria for the study were elderly and old age (60–90 years according to WHO) with confirmed test results of impaired walking and balance in patients with a history of chronic cerebral ischemia.

Patients with high spasticity (3 or more by the modified Ashworth scale), atrial fibrillation, infectious processes, impaired pain sensitivity, epilepsy, lower limb joint endoprosthetics, low scores (less than 24 b, corresponding to mild dementia) on the MMSE scale were excluded from the study.

2.2 Electromyostimulation

The duration of electrostimulation was designed for a 2-week inpatient stay (two courses of 5 days each) in addition to medication treatment. However, due to coronavirus restrictions, between 3 and 9 EMS procedures were performed (median 7). Data on the number of EMS treatments are shown in **Supplementary Table S2** of the supplementary materials. The procedure was always performed in the first half of the day. Patients were not warmed up in any way before the EMS procedure.

Patients in the control group did not receive electromyostimulation sessions. However, they received standard treatment.

Patients in the EMS group underwent lower limb stimulation in sinusoidal modulated current mode (not to be confused with high voltage pulsed current) using two single-channel Amplipulse-5DS stimulators (Russia) (**Figure 1A**). Carrying frequency of sinusoidal oscillations was 5 kHz, modulation frequency—50 Hz (**Figure 1B**). Amplitude of stimulation was set according to its peak tolerance by the patient. The range of amplitudes was from 10 to 40 mA, but the group average mean was 22–25 mA (**Supplementary Figure S1** of supplementary materials). Stimulation and rest periods amounted 4 and 6 s, respectively (**Figure 1B**). The total duration of one session was 20 min, 5 min each for stimulation of the anterior and posterior hip muscles (mm. semitendinosus, biceps femoris, quadriceps femoris) and shin (mm. triceps surae, tibialis anterior) (**Figure 1C**). The timing of the stimulation was chosen according to the protocol used by the cosmonauts. Wet electrodes (5 × 15 cm) of conductive rubber were used.

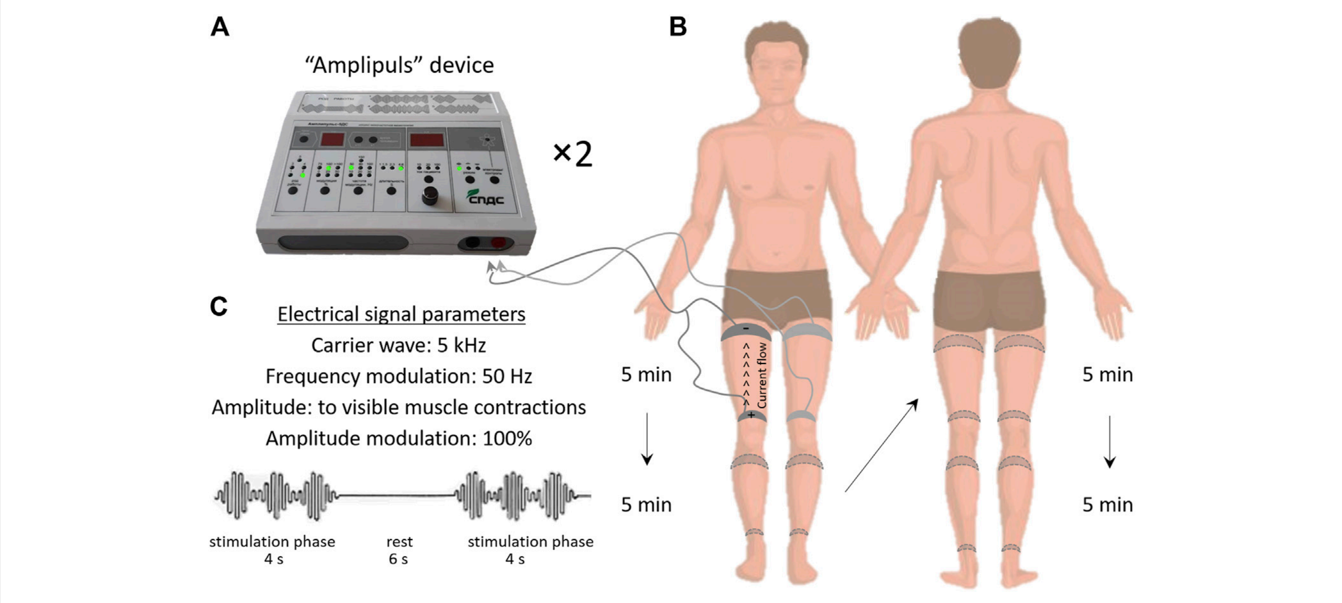

FIGURE 1 | Electromyostimulation protocol. General view of the Amplipulse-5DS stimulator **(A)**, electrical signal parameters **(B)**, electrode arrangement and order of stimulation of the lower limb segments **(C)**.

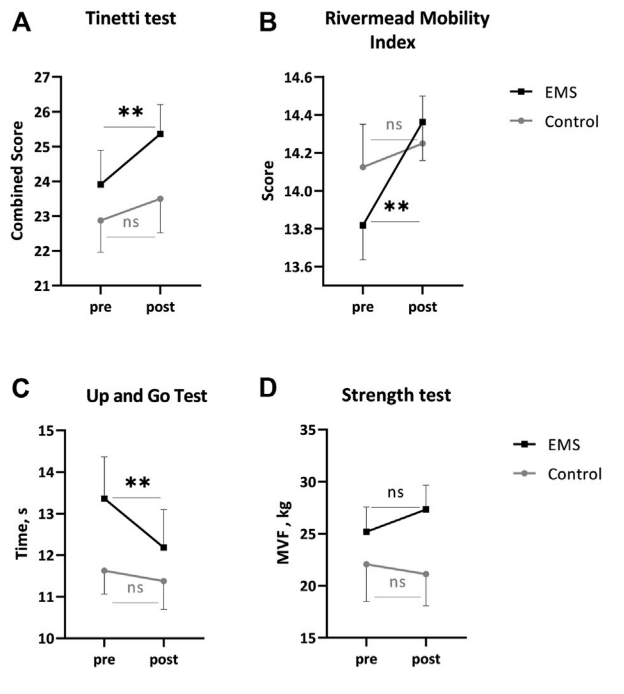

FIGURE 2 | Main indicators from the Tinetti test. **(A)**, Rivermead Mobility Index **(B)**, Up and Go **(C)**, Strength **(D)**. Mean ± SEM. $^{**}p < 0.002$ pre vs. post.

2.3 Study Design

Patients were interviewed and a battery of tests was performed twice: on admission to hospital and after the last EMS session. A battery of tests was conducted in the sequence below.

The Tinetti Scale (Scura and Munakomi, 2022) and *the Rivermead Mobility Index* (Williams, 2011) were used to determine the degree of activity of daily living.

The Timed Up and Go (2010) Test was performed according to a standard protocol (Kear et al., 2017). The patient had to get up from a chair, walk for 3 m, turn around, go back and sit down. The time over the distance was recorded.

Postural stability was assessed using the BioMera stabilography platform (BioMera LLC, Russia). The patients stood on the platform for 1 minute with their eyes open, and then another minute with their eyes closed. The fluctuations of the centre of pressure (CoP) were recorded. Path length (L), velocity of CoP movement (V) and statokinesiogram area (S) were analyzed. The Equilibrium Score (EQ), a dimensionless parameter assessing the ability to maintain equilibrium, was also calculated according to the formula: EquiScore = [1-(P-Psway)/12.5°]*100, where P-Psway is the maximum oscillation of the center of pressure in the sagittal plane, 12.5° is assumed to be the limit of stability for a normal individual (Chaudhry et al., 2004). To obtain the displacement of the center of gravity from the stabilogram, it was filtered with a 2nd order Butterworth high-pass filter that cut off frequencies above 0.85 Hz. The center of gravity was considered to be a point located at 55% of the height of the subject (Shishkin et al., 2019).

Muscle tone was assessed using MyotonPRO device (MyotonLTD, Estonia). The device applies mechanical impulses of stable strength and duration, and registers damped harmonic oscillations, from which viscoelastic properties of the studied tissue are calculated using a special mathematical algorithm (Schneider et al., 2015). The viscoelastic properties of mm. soleus, gastrocnemius lat and med, tibialis anterior, semitendinosus, biceps femoris, rectus femoris, and vastus lat were examined. During this examination, subjects were laying in prone or supine positions. In order to standardize the position of the lower limbs, rollers were placed under the knee and ankle joints. Standard parameters (Stiffness, Frequency, and Creep) were analyzed, the calculation procedure of which can be found elsewhere (Schneider et al., 2015).

The Strength test for the shin muscles was performed in the supine position using a specially designed tensometric pedal. The patient's leading leg was fixed in a position with the ankle, knee and hip joint angles of 90°. The patient made three attempts of peak plantar flexion—maximal foot pressure on the pedal (engaging the shin muscles, but not the hip muscles), the best result was recorded. The maximal voluntary force (MVF) developed by the lower leg muscles was analyzed.

2.4 Statistics

A two-way RM ANOVA with Sidak's posterior criterion was applied to determine significant statistical differences in the Tinetti, Rivermead, Up and Go, Strength, and muscle tone tests. The Mixed-effects model (REML), using the posterior Sidak test, was used to perform statistical analysis of stabilographic parameters (L, V, S). The Mann Whitney test was applied to determine statistical differences in the percentage difference in EQ between the EMS and control groups. The significance level is α = 0.05. All data are presented as Mean ± SEM.

3 RESULTS

3.1 Effects of Electromyostimulation on Patients' General Well-Being and Tolerance

The study found that the hip and shin electrostimulation procedure was well tolerated by all age groups of patients and no significant side effects were reported. A patient with giant Baker's cysts (anamnestic) presented with moderate pain in the knee of the affected limb during EMS was excluded from the study. Another patient had a minor hemorrhoidal bleeding once after the first treatment, which was caused by the drug combination. Thereafter, no bleeding was observed after correcting the therapy and the patient completed the course of therapy. Some patients experienced short-term moderate muscle pain, as after intensive physical activity for 1–2 days which was probably due to excessively high amplitude during stimulation.

Slight subjective improvement was noticed after 3-4 sessions. After a course of 8-9 sessions all the patients noticed a subjective improvement of postural stability, gait and ability to climb stairs more easily. It is also worth noting that the procedures were interesting and positive for the patients.

3.2 Questionnaires and Tests

The pre-study, groups scored 23.9 ± 0.9 (EMS) and 22.8 ± 0.9 (control) on the Tinetti test (**Figure 2A**). Post examination, the EMS group showed a significant improvement (+1.4 points, $p = 0.0045$), in contrast to the control group,

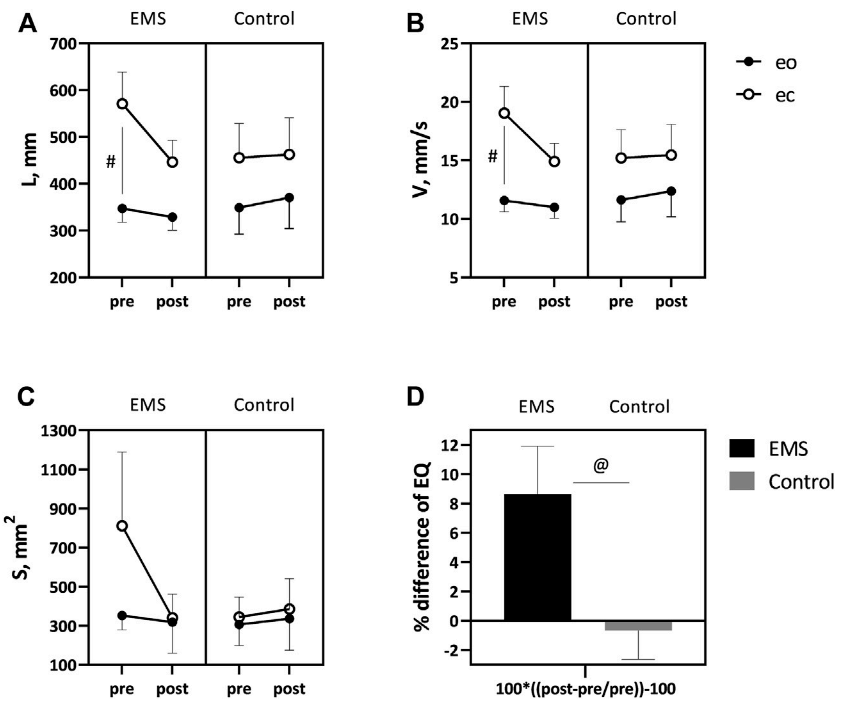

FIGURE 3 | Comparative data for length **(A)** and velocity **(B)** of the CoP and area of the statokinesiogram **(C)** in eyes open (shaded icons) and closed (not shaded icons), and also change in EQ **(D)** in the two patient groups. Mean ± SEM. # –p < 0.05 eyes open vs. eyes closed.at –p < 0.05 EMS vs. control.

TABLE 1 | Comparative table of the main parameters of skeletal muscle tone properties in two groups of patients.

	Stiffness		Frequency		Creep	
	Pre	Post	Pre	Post	Pre	Post
m. semitendinosus						
EMS	235,1	229,5	12,9	12,8	1,7	1,7
Control	249,6	236,3	14,2	**13,0***	1,5	1,6
m. biceps femoris						
EMS	286,4	276,4	15,4	15,2	1,3	1,4
Control	278,9	280,9	15,7	15,2	1,4	1,4
m. gastrocnemius lat						
EMS	282,4	290,8	14,5	15,5	1,5	1,4
Control	280,7	294,8	14,8	14,8	1,4	1,4
m. gastrocnemius med						
EMS	273,2	267,5	15,5	13,1	1,6	1,6
Control	273,1	273,6	12,9	13,1	1,5	1,5
m. rectus femoris						
EMS	270,6	277,6	13,4	13,9	1,5	1,4
Control	257,9	256,4	12,6	12,5	1,6	**1,7#**
m. soleus lat						
EMS	333,2	350,9	16,7	17,7	1,2	1,1
Control	**405,7#**	391,6	**20,1#**	19,7	**0,9#**	**0,9#**
m. soleus med						
EMS	341,4	335,1	16,4	16,6	1,1	1,2
Control	371,9	372,3	18,0	17,3	1,0	1,0
m. tibialis anterior						
EMS	387,5	393,9	19,4	19,5	0,9	0,9
Control	399,2	384,8	19,1	18,0	1,0	1,0
m. vastus lat						
EMS	279,8	281,7	13,9	14,7	1,5	1,4
Control	259,2	253,9	12,8	13,4	1,7	1,5

#–p < 0.05 EMS, vs. Control. *– p < 0.05 pre vs. post.

where no such improvement was observed (+0.6 points, p = 0.4).

The Rivermead Mobility Index showed a similar pattern of change (**Figure 2B**). The initial pattern (13.8 ± 0.1 in EMS and 14.1 ± 0.1 in control) changed to a significant increase post-EMS (+0.5 points, p = 0.0022). No significant changes were found in the control group (+0.1 points, p = 0.7).

The TUG test showed the following values before the study: 13.3 ± 1.0 s in the EMS and 11.6 ± 0.5 s in the control groups (**Figure 2C**). The EMS patients accelerated the distance run by 1.2 s (p = 0.0053) post-study. No significant changes were found in the control group (– 0.25 s, p = 0.7).

The maximal voluntary force was 25.1 ± 2.3 kg and 22.0 ± 3.5 kg in the EMS and control groups, respectively (**Figure 2D**), pre-study. No significant changes were recorded for MVF in two groups post-study.

3.3 Postural Stability

Post EMS course, patients visually improved postural stability, while no changes were observed in the control group.

L was 347.1 ± 29.2 mm in pre-study in the EMS group with eyes open, increasing significantly to 571.0 ± 67.9 mm with eyes closed (**Figure 3A**). In post-study, there was a slight downward trend in L in both open and closed eyes. In the control group, however, L with the open eyes was 349.1 ± 56.7 mm, increasing slightly to 455.7 ± 73.3 mm when the eyes were closed. No significant changes in L were observed after the study in the control group.

Similar trends were shown by V. Baseline V values in the EMS group amounted 11.5 ± 0.9 mm/s and 19.0 ± 2.2 mm/s for eyes open and closed, respectively (**Figure 3B**). Post-study, V was not significantly different from baseline values. In the control group, pre-study V values were 11.6 ± 1.8 mm/s for eyes open and 15.2 ± 2.4 mm/s for eyes closed. No significant changes in V were observed at the post-study period.

S in the EMS group was 352.8 ± 73.3 mm^2 in eyes open and 812.0 ± 377.1 mm^2 in eyes closed (**Figure 3C**). Post-EMS application, S showed a decreasing trend, which, however, was not significant. In the control group, S was 306.8 ± 107.5 mm^2 in eyes open and 345.0 ± 102.9 mm^2 in eyes closed. At the post-study, S was slightly higher than baseline values, 336.7 ± 161.0 mm^2 in eyes open and 385.6 ± 156.5 mm^2 in eyes closed.

The EQ, which is an integral indicator of an individual's postural stability, was calculated from the data obtained. Pre-study, the EQ was 80.0 ± 10.6 in the EMS group and 85.2 ± 5.8 in the control group out of a possible 100 points (**Figure 3D**). Post the EMS course, balance quality improved by 8.6% in the stimulation group (p = 0.04). No changes were registered in the control group.

3.4 Muscle Tone

Comparative data on the muscle tone are shown in **Table 1**. In the vast majority of cases, no significant changes in the studied parameters were found.

4 DISCUSSION

Thus, preliminary studies involving an initial small sample of neurological patients after EMS treatment with Russian currents support our hypothesis by 1) improvement in daily motor skills, a decrease in distance time and a trend towards increased muscle strength and 2) stabilisation of vertical stance. However, there is no change in muscle tone in patients after EMS 3). No significant changes in all parameters were recorded in the control group.

The application of EMS in patients with diseases of different genesis is not new and is actively used in medicine (Nussbaum et al., 2017; Silva et al., 2020). In particular, much of this research has been devoted to the treatment and countermeasure for elderly people (Evangelista et al., 2021), those with muscle weakness (Jones et al., 2016) or those in hypodynamic conditions (Wageck et al., 2014). In this study, we used Russian currents regimens analogous to use for cosmonauts, which should give the greatest effectiveness in a short period of time. We assumed that each patient would be able to receive 9–10 EMS procedures, but the coronavirus restrictions imposed reduced the number of sessions. We did not expect that the 6.3 procedures that our participants received on average would have such a significant effect in almost all tests and examinations performed. Previously it had been shown that 6 EMS procedures over 4 weeks had a positive effect on the 6-min walking test and muscle strength in patients, but in addition to the stimulation sessions the participants performed physical exercises (squats, lunges, biceps curl, chest press, butterfly reverse, reverse lunges, standing diagonal crunches, etc), which does not allow to make a direct conclusion on the results. In the vast majority of cases, however, there is a long duration of courses, ranging from several weeks to a year (Kemmler et al., 2018).

One of the most informative tests to assess the functional status of older adults is the Up and Go test (Freiberger et al., 2013). In a study involving elderly volunteers, it was shown that a 9-week EMS (24 training sessions) improved the time to pass the TUG test by 16.4% (Kern et al., 2014). In our study, a similar improvement was 9.0%, but it was achieved in a shorter time.

In the Nishikawa study, lower extremity stimulation for 12 weeks in elderly patients with dementia improved muscle strength (p = 0.008) and postural stability (p = 0.007), in contrast to the control group where these characteristics worsened (Nishikawa et al., 2021). In our study, an improvement in postural stability in patients after EMS treatment was reliably recorded at an earlier time point. However, in contrast to Nishikawa's work, no significant increase in muscle strength was recorded in our study, but a clear upward trend in MVFwas observed in the EMS group. It is likely that a significant increase in strength requires a bigger amount and/or duration of EMS sessions.

A factor that we did not take into account in this study was life satisfaction and general emotional mood while in hospital. We also did not record patients' daily motor activity. There is evidence that the psycho-emotional state of elderly patients may have an effect on their motor function (Beheydt et al., 2014). However, we believe that since the main difference between the groups was the use of EMC, it is more likely that this fact can explain the results.

It also needs to be mentioned that for some baseline parameters, the EMS and control groups were slightly different, although there were no significant differences. For example, TUG time was 1.7 s better in the control group (13.3 s EMS vs. 11.6 s control). According to the classification (Bischoff et al., 2003), community-dwelling elderly women should be able to complete the TUG test in 12 s or less, which correlates with the control group. The TUG test values of the group with EMC, although significantly improved after a course of Russian currents, still averaged more than 12 s. However, according to the classification of the American College of Rheumatology (2010), test results of 10–20 s are considered normal for older adults.

It is also important to note that this article is a preliminary report of the results. The small number of participants so far allows us to draw intermediate conclusions about the beneficial effects of short courses of modulated EMS. We plan to increase the number of participants in each group as well as to add another group with sham stimulation to offset the effects of the procedure itself, such as the application of electrodes, the extra attention of the nursing staff and the feeling of current. Research into the effects of EMS on the motor performance of patients with stance and walking disorders will continue and the final results will be published at a later date.

5 CONCLUSION

It can be concluded that stimulation of the hip and shin muscles with Russian (Kotz) currents has a positive effect on the motor system of neurological patients of advanced age. Significant effects with a course of short duration indicate the promise of this EMS treatment regimen. However, the preliminary character of the data requires further research to form significant conclusions.

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 bioethical committee of the Solovyov RPC of DZM (protocol no. 49 of 30 July 2021). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

LA developed study design, processed data, wrote manuscript. MA participated in study design, conducted research, worked with patients. NS processed data and made a revision of the manuscript. AG participated in study design, conducted research, worked with patients. AG participated in study design, revised manuscript. ET made a revision of the manuscript and was the study supervisor. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphys.2022.921434/full#supplementary-material

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Implementation of exercise countermeasures during spaceflight and microgravity analogue studies: Developing countermeasure protocols for bedrest in older adults (BROA)

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Significant progress has been made in the development of countermeasures to attenuate the negative consequences of prolonged exposure to microgravity on astronauts' bodies. Deconditioning of several organ systems during flight includes losses to cardiorespiratory fitness, muscle mass, bone density and strength. Similar deconditioning also occurs during prolonged bedrest; any protracted time immobile or inactive, especially for unwell older adults (e.g., confined to hospital beds), can lead to similar detrimental health consequences. Due to limitations in physiological research in space, the six-degree head-down tilt bedrest protocol was developed as ground-based analogue to spaceflight. A variety of exercise countermeasures have been tested as interventions to limit detrimental changes and physiological deconditioning of the musculoskeletal and cardiovascular systems. The Canadian Institutes of Health Research and the Canadian Space Agency recently provided funding for research focused on Understanding the Health Impact of Inactivity to study the efficacy of exercise countermeasures in a 14-day randomized clinical trial of six-degree head-down tilt bedrest study in older adults aged 55–65 years old (BROA). Here we will describe the development of a multi-modality countermeasure protocol for the BROA campaign that includes upper- and lower-body resistance exercise and head-down tilt cycle ergometry (high-intensity interval and continuous aerobic exercise training). We provide reasoning for the choice of these modalities following review of the latest available information on exercise as a countermeasure for inactivity and spaceflight-related deconditioning. In summary, this paper sets out to review up-to-date exercise countermeasure research from spaceflight and head-down bedrest studies, whilst providing support for the proposed research countermeasure protocols developed for the bedrest study in older adults.

KEYWORDS

spaceflight, bedrest, cardiovascular, musculoskeletal, exercise countermeasures, aging

Introduction

Spaceflight causes marked changes to the musculoskeletal and cardiovascular systems like those observed during prolonged inactivity. Accordingly, even early human spaceflight incorporated exercise countermeasures aimed at preventing physical deconditioning (Hackney et al., 2015). Exercise equipment during early short-duration flights (Gemini and Apollo missions) was small and simple in design (e.g., bungee exerciser) (Hackney et al., 2015). Aerobic exercise equipment was available during Space Shuttle flights, and exercise was recommended without prescribed intensity or duration for every second or third mission day (Scott et al., 2019); however, some shuttle astronauts did not exercise while in orbit (Edgerton et al., 1995). More advanced equipment with greater loading capabilities was utilized during longer-duration missions, and more recently aboard the International Space Station (Hackney et al., 2015). Current International Space Station (ISS) exercise hardware includes a cycle ergometer (CVIS), treadmill (T2), and advanced resistive exercise device (ARED) (Korth, 2015). Astronauts on-board the ISS are typically allocated 2.5 h of total exercise time per day, which consists of 1.5 h of resistance training and 1 h aerobic training (Loehr et al., 2015). Importantly, the actual time spent performing exercise each day is much less (Fraser et al., 2012), as the allotted time also includes equipment set-up and stowage, and personal hygiene (Loehr et al., 2015).

Six-degree head-down bedrest (HDBR) is widely used as an experimental model to simulate the effects of microgravity and evaluate the effectiveness of different exercise countermeasures before implementing them during spaceflight; however, there are some notable differences between spaceflight and HDBR related to fluid shifts (see Table 1), spinal dysfunction and radiation exposure (Hargens and Vico, 2016). Different exercise countermeasures have been tested during HDBR, such as aerobic (Stremel et al., 1976; Greenleaf et al., 1989) or resistance training protocols (van Duijnhoven et al., 2010b), novel exercise modalities (Kramer et al., 2017a), and combinations of exercise with fluid loading (Shibata et al., 2010), artificial gravity (Watenpaugh et al., 2000; Iwasaki et al., 2005; Schneider et al., 2009) or whole-body vibration (van Duijnhoven et al., 2010b; Coupé et al., 2011; Guinet et al., 2020). In addition to aerospace medicine research, bedrest experimental models are also useful for examination of the rapid physiological deconditioning that occurs during prolonged hospitalization and inactivity on Earth.

An important disconnect exists between those who typically participate in HDBR studies and astronauts. The median age of bedrest study participants is 24.5 years (inter-quartile range: 22.4–34.0 years) (Ried-Larsen et al., 2017), whereas the mean age of astronauts for first and last flights are 40.9 years (maximum: 58.8 years) and 45.3 years (maximum: 61.3 years, excluding John Glenn at 77.3 years), respectively (Kovacs and Shadden, 2017). Both spaceflight and HDBR studies are infrequent, with very limited investigations of older adults. Accordingly, an important knowledge gap exists on the effects of HDBR, and exercise countermeasures on middle-aged and older adults.

The purpose of this review is to provide an overview of the musculoskeletal and cardiovascular responses to spaceflight, bedrest, and aging, as well as how exercise can mitigate deconditioning in these systems. There is a lack of knowledge regarding HDBR-induced deconditioning in older adults, and we draw attention to knowledge-gaps for physiological systems explored in this review. Furthermore, we introduce the BROA study and discuss the rationale and development of a multimodal

TABLE 1 Comparison of the effects of spaceflight and head-down bedrest (HDBR) on blood volume (+: increased, –: decreased). Adapted and modified from Diedrich et al. (2007).

	Spaceflight	HDBR
Headward fluid shift	+	+
Hunger	–	+/-
Thirst	–	+/-
Diuresis	+/-, (explained above)	+
Renal response to fluid & salt	Reset	Preserved
Salt retention	+	Unknown
Atrial natriuretic peptide	Reset to lower levels	+
Mechanical compression of thorax	–	Maintained
Central venous pressure	–	Transiently increased
Radiation	+	NA

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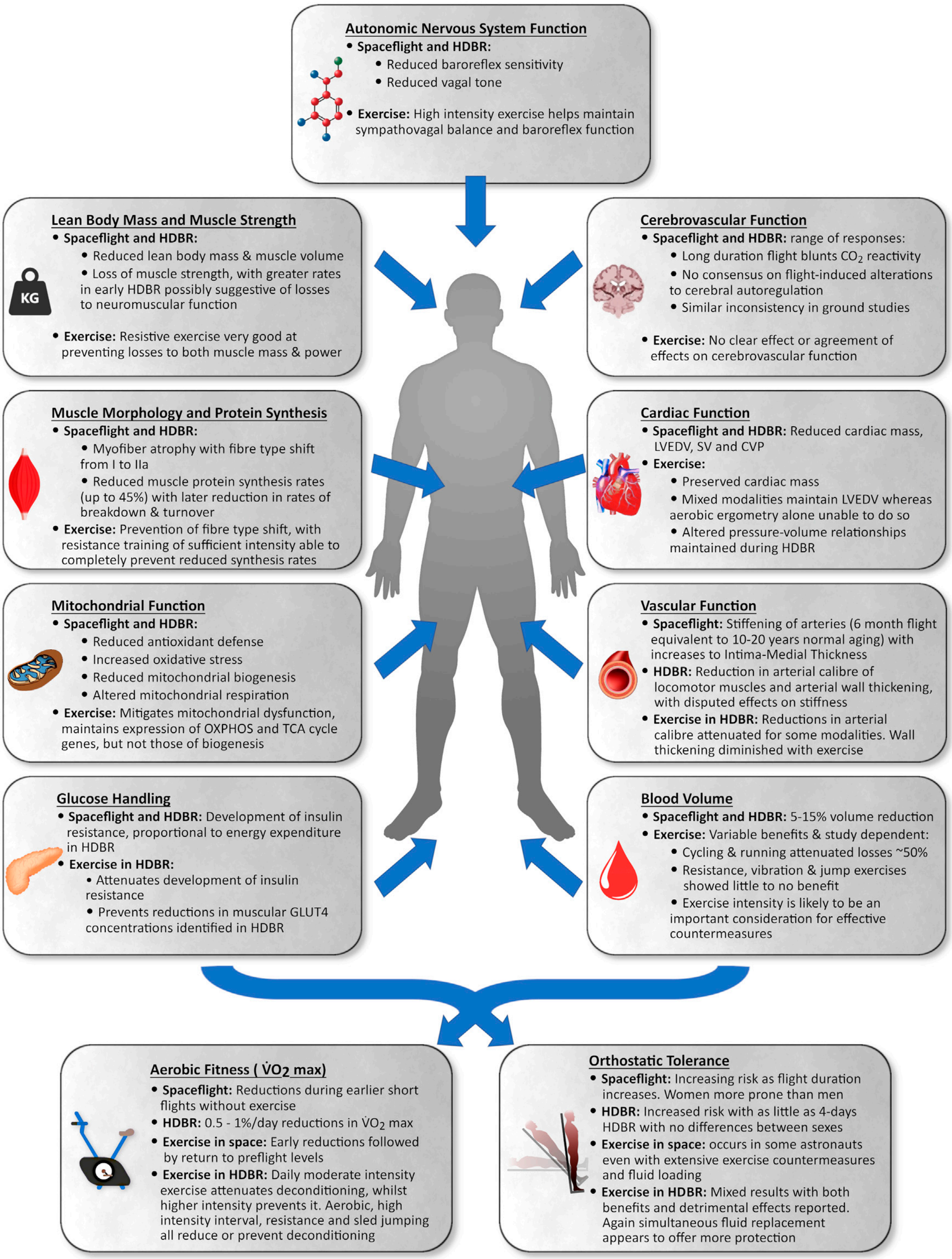

FIGURE 1
Summary of the impact of spaceflight and head-down tilt bedrest (HDBR) in young to middle-aged adults and the impact of exercise as a countermeasure on various portions of the cardiovascular and musculoskeletal system.

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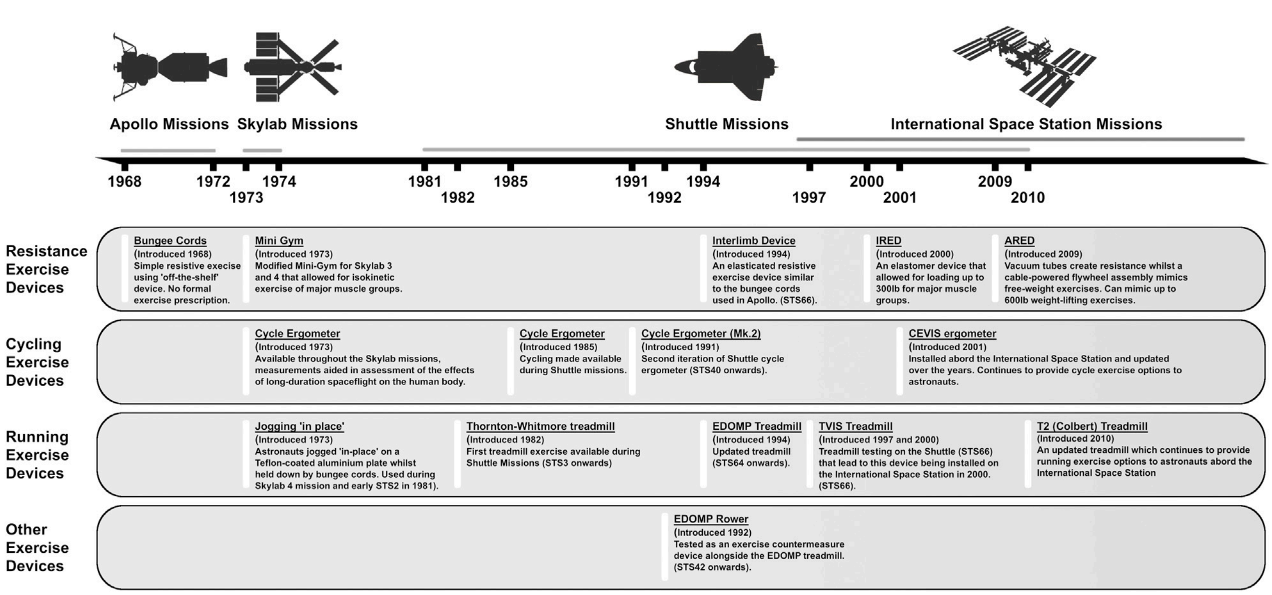

FIGURE 2
Timeline overview of exercise equipment available on Apollo, Skylab, Shuttle and ISS missions from 1968 to present day.

exercise countermeasure protocol that will be utilized during this two-week HDBR in older adults aged 55–65 years. Figure 1 provides an overview of the knowledge reviewed in this paper.

Exercise countermeasures employed during the WISE-2005 study

We will refer to this study throughout this review. The WISE-2005 study was a 60-day HDBR that combined supine squats (4 sets of 7 maximal repetitions) and calf presses (4 sets of 14 maximal repetitions) 2–3 times/week, with aerobic vertical treadmill exercise in lower-body negative pressure (LBNP) calibrated to 1.0x body weight 2–4 days/week with steps up to 80% peak oxygen uptake ($\dot{V}O_2$).

Lean body mass and muscle strength

Spaceflight

Gravitational unloading during spaceflight results in loss of lean body mass and muscle strength. During early Skylab missions, lower-limb muscle volume estimated by leg circumference (↓~5%) and muscle strength (↓~25%) were reduced (Thornton and Rummel, 1977). Subsequently, studies confirmed muscle losses following short-duration flights in the major muscles of the lower limbs (LeBlanc et al., 1995; Akima et al., 2000) and trunk (LeBlanc et al., 1995). These losses led to reductions in trunk (↓23%) and lower-body strength (↓12%) (Greenisen et al., 1999). The introduction of regular exercises, improvements in exercise equipment and introduction of new exercise modalities (Figure 2), as well as increases to exercise duration (~30 W-mins/day during Skylab 2 to ~70 W-min/day during Skylab 4) attenuated but did not prevent muscle losses during Skylab missions (Thornton and Rummel, 1977). Even during early missions to the ISS, losses to lower-limb muscle mass and strength persisted, despite regular exercise countermeasures (Trappe et al., 2009; Gopalakrishnan et al., 2010). It was not until the introduction of the T2 and ARED devices that astronauts were able to maintain proportional lean body mass during their flights (Smith et al., 2012). Most recently, NASA's SPRINT study concluded that a combination of resistance training (3 days/week) and a mix of high-intensity interval and continuous aerobic training (6 days/week) was just as effective as standard countermeasure regimes at preventing loss of lean leg mass and strength, requiring 33% less time to complete (English et al., 2020). A recent systematic review determined that current countermeasure exercises, particularly resistance exercises using newer equipment, are crucial in preventing muscle losses during spaceflight, although complete prevention is still not possible (Moosavi et al., 2021).

Bedrest studies

Muscle size and strength losses during bedrest studies are similar to those induced by spaceflight (Narici and de Boer, 2011), with lower-limb and trunk musculature generally affected more than upper-limb muscles (Winnard et al., 2019). Both muscle size and strength followed a nonlinear logarithmic decay during bedrest, with higher rates of change initially that slow over time (Marusic et al., 2021). The ratio of proportional strength loss to muscle atrophy was elevated initially during bedrest before plateauing by ~35 days, suggesting factors other than muscle atrophy largely contribute to early reductions in strength; however, the vast majority of reductions to muscle strength during bedrest can be explained by muscle atrophy (Marusic et al., 2021).

During a 17-week horizontal bedrest, an exercise countermeasure consisting of progressive upper- (3 days/week) and lower-body (3 days/week) resistance exercises starting at 2 sets and increasing to 6 sets by the end of the campaign, with each set consisting of 11 repetitions at 74% of a 1-repeition maximum, and heel raises (6 days/week), with the same set and repetition scheme as the other exercises on lower-body days and a 5-set, 20 repetition protocol at 58% of 1-repeition maximum on upper-body days, attenuated but did not prevent muscle volume losses, despite improving isotonic strength (Shackelford et al., 2004). Muscle atrophy and strength losses during HDBR were also attenuated by resistive vibration exercise (Rittweger et al., 2010), or aerobic treadmill exercise with body weight loading (Cavanagh et al., 2016). In the WISE-2005 study, again muscle strength increased whilst volume losses were attenuated in exercisers during 60 days of HDBR (Trappe T. A. et al., 2007). A comparison of the type of exercise device (traditional resistance vs. flywheel) found little difference in muscle outcomes between groups (Ploutz-Snyder et al., 2018). Only one set of exercise countermeasures at NASA's Flight Analogues Research Unit (FARU) during HDBR prevented volume losses completely in targeted muscles (3 days/week of resistance exercises comprising 3 sets of 12 reps at 10-repetition maximums loading for supine squat, leg press, heel raise, and leg curl, and alternating days supine cycling switching between continual aerobic at 80% of peak $\dot{V}O_2$ or interval training up to maximal effort) when delivered over a 14-day HDBR in which muscle strength also increased (Ploutz-Snyder et al., 2014). Improvements in participants' technique and neural adaptation to resistance training may account for the improvements in strength without increases to muscle volume. However, the clear benefit of exercise on both volume and strength compared to control participants clearly supports efficacy of exercise training during HDBR and periods of immobility. Furthermore, the frequency of the intervention is important; over 90 days of HDBR, supine squats (4 sets of 7 maximal repetitions) and calf presses (4 sets of 14 maximal repetitions) every third day (starting HDBR day 5) only attenuated reductions to lower-limb muscle cross-sectional area (CSA; ↓25.6% in control vs. ↓17.3% in exercise) (Rittweger et al., 2005), which is a substantially smaller benefit compared to the aforementioned studies. In summary, exercise, particularly regular resistance exercise, can attenuate muscle volume losses and even improve strength during both supine and HDBR.

Older adults

Increasing age may accelerate the rate of bedrest-induced muscle loss (English and Paddon-Jones, 2010). Reductions in lean body mass and strength were found following as little as 10 days of sedentary bedrest in older adults (Kortebein et al., 2008; Coker et al., 2015). Comparison of 14-day supine bedrest in older (55–65 years) vs. younger (18–30 years) adults identified more detrimental effects on muscle power in the older age group, identifying important differences in their responses to disuse (Rejc et al., 2018). We identified one seven-day bedrest study in older adults with a 2000 steps/day exercise intervention, but the countermeasure did not prevent loss of muscle mass or strength (Arentson-Lantz et al., 2019). Therefore, formal evaluation of exercise countermeasures in older adults is paramount, especially given that loss of muscle mass and strength results in a significant reduction in functional ability (Kortebein et al., 2008; Coker et al., 2015), potentially leading to decreased ability to perform activities of daily living and a loss of independence.

Muscle morphology

Spaceflight

Exposure to microgravity reduces skeletal muscle size, function, and metabolic capacity by inducing changes at the myocellular level. Spaceflight-induced fiber type shifts from type I (oxidative) to type IIa (oxidative/glycolytic) are known to occur during both short- (Edgerton et al., 1995; Zhou et al., 1995; Widrick et al., 1999) and long-duration flights (Trappe et al., 2009; Fitts et al., 2010), with concomitant increases in co-expressing fibers (Widrick et al., 1999, Widrick et al., 2001; Trappe et al., 2009). Combined with reductions in myofiber CSA, as noted in the vastus lateralis (VL) (Edgerton et al., 1995) and soleus muscles (Widrick et al., 1999, Widrick et al., 2001; Trappe et al., 2001), spaceflight causes serious detrimental effects to muscle function. This can lead to greater susceptibility to muscle fatigue, decrease of muscle strength and speed of muscle contraction in astronauts potentially limiting their work capacity during spaceflight. Despite very limited available data in astronauts due to concerns regarding complications from repeated muscle biopsies, we do know that these changes occur even when aerobic exercise or resistance exercise devices, such as iRED (See Figure 2), are available (Trappe et al., 2009). Evidence suggests that the magnitude of myofiber losses are inversely related to the intensity of in-flight aerobic exercise (Fitts et al., 2010), but definitive assessment of optimal countermeasure protocols to combat these losses does not yet exist.

Bedrest studies

As with spaceflight, detrimental morphological muscle changes occur during bedrest. Previous studies identified reductions in CSA of type IIa myofibers in VL muscle following 10-day supine bedrest (Standley et al., 2020), and of all myofiber types in the VL after 35- and 37-day HDBR (Andersen et al., 1999; Borina et al., 2010; Brocca et al., 2012). Following 60-day HDBR, CSA of type I and IIa myofiber types in soleus and VL muscles were reduced (Salanova et al., 2014). Over a 122-day HDBR campaign, Ohira et al. observed progressive reductions to type I myofiber CSA in the soleus, with 12% losses at the halfway point, and 39% losses by the end of the study (Ohira et al., 1999). These reductions in CSA tended to be accompanied by changes in myofiber type (Ohira et al., 1999). Shifts away from type I and towards type IIx co-expressing myofibers was noticed in protein analyses of the VL muscle following 35-day HDBR (Borina et al., 2010; Brocca et al., 2012), whilst Andersen et al. identified corroborating changes in mRNA expression of myosin heavy chain protein genes without difference in protein levels following 37 days of HDBR (Andersen et al., 1999). Decreases in the proportion of type I myofibers in both the soleus and VL muscles, and an increase in the proportion of co-expressing myofibers were noted in the 60-day HDBR campaign (Salanova et al., 2014). Myofiber atrophy commences soon after the initiation of bedrest and can gradually progress for at least 4 months (Ohira et al., 1999), but beyond this time point no data exists. Therefore, similar morphological changes occur between spaceflight and bedrest studies.

Regarding exercise countermeasures, the combination of aerobic exercise with LBNP and resistance training, resistance exercise and vibration, or just resistance exercise during 60 days of HDBR, maintained myofiber CSA and further increased the CSA of type IIa myofibers in both soleus and VL muscles (Trappe S. et al., 2007; Salanova et al., 2008, Salanova et al., 2014). Resistance training 2–3 days/week during an 84-day HDBR campaign (4 sets of 7 maximal supine squats) preserved type IIa myofiber composition and size in VL muscle, and type IIa and type I myofiber composition in soleus muscle (Trappe et al., 2004; Gallagher et al., 2005). However, both the exercise and control groups in this study displayed an increased proportion of co-expressing myofibers in these muscles (Trappe et al., 2004; Gallagher et al., 2005). More recently, Blottner et al. (2020) reported an almost completely protective effect of head-down sled jumping for both type I and II fibers' CSA in the soleus muscle, with no myofiber shifts in the VL despite a downward trend in the proportion of type I fibers in the soleus. The high load jumping (80%–90% bodyweight) provided over 48 exercise sessions during the 60-day campaign was an effective countermeasure for HDBR-induced muscular changes noted in the VL and soleus of control participants (Blottner et al., 2020).

Therefore, exercise attenuates, and may even prevent, the detrimental effects of HDBR on muscle morphology and serves as an analogue to spaceflight studies. Exercise programs during HDBR have the capacity to counteract some of the detrimental effects at the myocellular level; however, these studies focus on muscles of the lower limb, and countermeasure programs must be more holistic.

Older adults

Muscle mass and strength generally diminish with age, with concomitant myofiber atrophy (Lexell et al., 1988; Lexell and Taylor, 1991). Myofiber atrophy experienced with aging is exacerbated by physical inactivity (Arentson-Lantz et al., 2016; Pišot et al., 2016) and were not attenuated with a 2000-daily steps intervention (Arentson-Lantz et al., 2019). Given that older adults typically have less muscle, and it declines more quickly with inactivity, dedicated studies are needed to determine optimal exercise countermeasures in this special population to prevent muscle wasting during periods of immobility.

Muscle protein synthesis and breakdown

Spaceflight

Muscle protein mass represents the net balance between rates of muscle protein synthesis (MPS) and muscle protein breakdown (MPB), whose turnover rates are constantly in a dynamic state of adjustment, allowing the body to maximize protein balance when facing limited supply of amino acids (Stein, 1999; Morais et al., 2018). On immediately entering microgravity, nausea, vomiting and space motion sickness lead to stress-induced reductions in energy and protein intake, as well as negative nitrogen balance (Stein et al., 1993, Stein et al., 1996; Stein and Schluter, 1994). This is followed by an abrupt increase in MPB by flight day 3, and reductions to both MPS and MPB by flight day 6 (Ferrando et al., 2002), which supports historical measurements of negative nitrogen balance during Skylab missions (Whedon et al., 1977). Following the initial dynamic response to environmental changes, astronauts' muscle protein turnover appears to be reduced (Stein et al., 1999a; Ferrando et al., 2002). Indeed, there is decreased whole body protein synthesis during long-duration spaceflight (Stein et al., 1999a) and short-duration missions (Ferrando et al., 2002). In the later study, reduced MPB was also observed based on the urinary excretion of 3-methyl-histidine (Ferrando et al., 2002). These results suggest physiological adaptation to a state in which the body cannot maintain MPS at the same rate in skeletal muscle, likely due to reductions in activity and muscle loading, as well as an energy deficit. Therefore, exercise plays an important role in stimulating muscle protein turnover after the first days of spaceflight and exemplifies the need for adequate exercise countermeasures for astronauts. To the best of our knowledge the effect of aerobic and resistance exercise on muscle protein kinetics during spaceflight is yet to be studied.

Bedrest studies

Bedrest without countermeasures has detrimental effects on muscle protein kinetics. Fourteen-day (Ferrando et al., 1996) and 21-day HDBR (Symons et al., 2009) both reduced MPS without changing MPB. Few investigations have studied MPS and MPB during bedrest with exercise countermeasures. There is some evidence to suggest exercise is protective of muscle protein kinetics, as resistive knee extension exercises (3 sets of 10–12 repetitions/day with progressive daily loading) maintained lower-body MPS during 14-day HDBR (Ferrando et al., 1997). Sedentary bedrest studies consistently show muscle protein changes are induced by lower MPS rates whereas protein breakdown seems to be affected to a lesser degree (Ferrando et al., 1996; Symons et al., 2009). Exercise, especially resistance type mitigates such loss, but it needs to have a progressive overload nature to achieve protective effects on MPS and MPB (Ferrando et al., 1997). Further research in this field focusing on muscle protein metabolism, including changes in kinetics and cellular signalling proteins, and the impact of countermeasure exercises during HDBR are required to fully understand how exercise may provide beneficial results in immobile or weightless individuals.

Older adults

Aging is associated with anabolic resistance to MPS in the ambulatory state as well as during bedrest, which predisposes older adults to increased muscle atrophy compared with their younger counterparts. Older adults appear to be at a higher risk for muscle loss during bedrest, as two-week supine bedrest increased anabolic resistance to a standardized meal four times more in older (~59 years) compared to younger adults (~23 years) (Biolo et al., 2017). Similarly, older adults (~66 years) had diminished post-prandial fractional protein synthesis rates and elevated markers of muscle proteolysis with reduced lower-limb muscle mass and strength following five days of supine bed rest, while younger adults (~22 years) were largely unaffected (Tanner et al., 2015). Exercise can combat impaired MPS in older adults (Dickinson et al., 2013), but the efficacy of exercise countermeasures to preserve MPS in older adults during bedrest has not been evaluated.

Mitochondria

Spaceflight

Mitochondria are implicated in several important cellular functions, including energy transduction, activation of apoptotic signaling, and regulation of reactive oxygen species (ROS) production (Hepple, 2016). These processes modulate a wide variety of cell signaling pathways, including those associated with muscle atrophy (Romanello et al., 2010). Spaceflight leads to increases in mitochondrial ROS production whilst also altering expression of genes related to adenosine triphosphate generation pathways in human fibroblasts (da Silveira et al., 2020). Within the muscle, no change in succinate dehydrogenase activity, a marker of myofiber oxidative capacity was found in the VL muscle from astronauts after a 11-day spaceflight (Edgerton et al., 1995). After a six-month spaceflight incorporating iRED, CEVIS and treadmill running countermeasures between 2002–2005 (Figure 2), the activity of cytochrome oxidase of complex IV of the electron transport chain was reduced by 59% in type I fibers of both soleus and gastrocnemius muscle of astronauts performing less treadmill training (<100 min s/week) compared to no change in those running for longer (Fitts et al., 2013). There was no change in these enzyme activities in type IIa fibers, nor were there any changes to activity of mitochondrial enzymes β-hydroxyacyl-CoA dehydrogenase and citrate synthase (CS), representing β-oxidation and tricarboxylic acid (TCA) cycle activity respectively, in soleus or gastrocnemius muscle independent of running duration (Fitts et al., 2013). This may be due to the high heterogeneity in the activity of given enzymes within a particular fiber, muscle type and the low number of subjects studied (Fitts et al., 2013). Nevertheless, the activity of β-hydoxyacyl-CoA dehydrogenase and CS was comparatively higher in soleus type I fibers of the high-intensity treadmill group post-flight (Fitts et al., 2013). It seems that appropriate in-flight exercise programs (duration, mode, intensity) can stimulate muscle aerobic enzyme activity (Fitts et al., 2013) which is important for maintenance of normal mitochondrial function. However, the role of in-flight exercise on muscle mitochondrial function requires further investigation.

Bedrest studies

After 14 days of supine bedrest, protein levels of PGC-1a, a master regulator of mitochondrial biogenesis (Popov, 2020), and Sirt3, a protein involved in mitochondrial activity (Vassilopoulos et al., 2014), were significantly reduced (Buso et al., 2019). Similarly, expression of eight subunits of CI, CII, CIV, and CV was reduced in addition to two subunits of the TIM/TOM complex (Buso et al., 2019), which is important for protein translocation through the mitochondrial membrane (Gomkale et al., 2021). After 10 days of supine bedrest, RNA-sequencing revealed reduction in the expression of genes involved in the TCA cycle and mitochondrial respiration measured in permeabilized myofibers, whilst ROS production increased, likely driven by reductions in mitochondrial content (Standley et al., 2020). Similar findings were observed after 21 days of supine bedrest and HDBR, where significant reduction in adenosine diphosphate-stimulated mitochondrial respiration and maximal aerobic capacity were found in VL muscle of young subjects (Kenny et al., 2017; Salvadego et al., 2018). Following 60 and 84 days of HDBR, gene expression analysis of VL and soleus muscle showed reduced expression of genes involved in TCA cycle, oxidative phosphorylation, lipid metabolism and mitochondrial biogenesis (Salanova et al., 2015; Irimia et al., 2017; Fernandez-Gonzalo et al., 2020).

With regard to exercise countermeasures, participants completing resistance (squats and heel raises) vibration (25 Hz) exercise training on five days during 21-day HDBR did not exhibit alterations in mitochondrial respiration (Kenny et al., 2017). Resistance exercise (squats) 2–3 days/week during 84 days of HDBR was not enough to preserve gene expression of some mitochondrial biogenesis markers (Irimia et al., 2017). However, resistance exercise intervention was able to offset alteration of gene expression related to oxidative phosphorylation, TCA cycle and fatty acid oxidation (Fernandez-Gonzalo et al., 2020). Finally, rehabilitation comprising a multimodal exercise program following 14 days of supine bedrest restored the protein levels of PGC-1a and Sirt3, and CS activity (Buso et al., 2019). However, only protein levels of complexes CII and CIII recovered to baseline values, whereas the expression of CV did not recover after the rehabilitation period (Buso et al., 2019). The exercise program upregulated genes involved in oxidative phosphorylation and TIM/TOM complex (Buso et al., 2019). These findings suggest that exercise during or immediately following bedrest can mitigate some of the negative effects of inactivity on mitochondrial function and other metabolic markers.

Older adults

Short duration supine bedrest (10 and 14 days) in older subjects reduced protein levels of almost all oxidative phosphorylation complexes (CI, CII, CIII, CIV, and CV) (Buso et al., 2019; Standley et al., 2020). Additionally, it reduced activity of CS, which is often used as marker of mitochondrial content (Buso et al., 2019). These detrimental effects were far greater than those identified in younger adults participating in the same study. Therefore, older populations (55–65 years) respond differently compared to younger populations, with a far greater impact of bedrest on mitochondrial function. However, so far as we are aware, no studies have yet investigated the effects of countermeasure exercises on mitochondrial function in older adults during HDBR.

Changes in cardiac function

Spaceflight

During early human space exploration, limited or non-existent availability of exercise modalities resulted in marked changes in cardiac function despite the relatively short exposure to microgravity. Following Apollo, Spacelab, and early Space Shuttle missions supine resting heart rate (HR) was increased in astronauts upon returning to Earth (Hoffler and Johnson, 1975; Bungo et al., 1987; Buckey et al., 1996b) whereas supine cardiac output ($\dot{Q}$) and stroke volume (SV) were decreased (Hoffler and Johnson, 1975; Bungo et al., 1987; Levine et al., 1996). Heart size (Hoffler and Johnson, 1975), left ventricle end diastolic volume (LVEDV) (Henry et al., 1977; Bungo et al., 1987) and LV mass (Perhonen et al., 2001a) were also reduced post-spaceflight. In microgravity, central venous pressure (CVP) is lower than supine posture, but the enhanced transmural pressure gradient increases cardiac filling (Buckey et al., 1996a; Lawley et al., 2017) resulting in increased SV and cardiac output while in space (Norsk et al., 2015; Hughson et al., 2017).

The extent of cardiac changes with longer duration space missions is influenced by the overall reduction in daily energy expenditure (Fraser et al., 2012) balanced to variable degrees by exercise countermeasures (Moore et al., 2010; Fraser et al., 2012). Immediately following long-duration spaceflight missions on Mir space station, reduced SV, ejection fraction and percent fractional shortening were observed (Martin et al., 2002). Currently, the combination of in-flight aerobic exercise and resistance training appears sufficient to maintain cardiac function assessed *via* heart-rate responses to normal daily living (Fraser et al., 2012). Transient prolongation of left ventricular ejection time post-flight and slightly increased resting HR (Hughson et al., 2012), appear to resolve quickly (Vandeput et al., 2013), consistent with adequate cardiac protection from current ISS countermeasure activities. Furthermore, maintenance of seated SV and $\dot{Q}$ (Hughson et al., 2012), and cardiac mass and LVEDV (Abdullah et al., 2018) following 4–6 months of spaceflight support this conclusion. It should be noted that left atrial volume increased in astronauts performing these same exercise countermeasures; however, this resolved quickly post-flight and did not result in any change in atrial function or increase in supraventricular arrhythmogenic potential (Khine et al., 2018). Importantly, with more advanced equipment and adequate exercise duration and intensity, cardiac function following spaceflight appears to be protected.

Bedrest studies

HDBR induces changes to cardiac function that are like those identified during spaceflight. Elevated resting supine HR following HDBR of 35 days (Hastings et al., 2012) and 60 days (Edgell et al., 2007), and reduced resting supine SV in studies of greater than two weeks (Levine et al., 1997; Perhonen et al., 2001a; Kozàkovà et al., 2011; Hastings et al., 2012), indicate significant cardiac consequences of the HDBR intervention. Similarly, losses to cardiac mass (Levine et al., 1997; Perhonen et al., 2001a; Kozàkovà et al., 2011), reductions to LVEDV (Levine et al., 1997; Perhonen et al., 2001a; Kozàkovà et al., 2011), and decreased LV distensibility contribute to a loss of cardiac function (Levine et al., 1997; Kozàkovà et al., 2011), with LV untwisting also noted to slow (Dorfman et al., 2008). The noted fall in CVP in spaceflight was also observed during HDBR at both the 4 h (Fischer et al., 2007) and two-week time points (Levine et al., 1997).

The intensities and modes of aerobic and resistance countermeasures during HDBR in combination with the subjects' activity levels prior to bedrest contribute to observations of variable cardiac responses (Dorfman et al., 2007; Shibata et al., 2010; Yang et al., 2011; Hastings et al., 2012; Li et al., 2017; Scott et al., 2018; Greaves et al., 2019). When compared to control participants, countermeasure exercises during the WISE-2005 study maintained HR, SV, and LVEDV and cardiac mass during HDBR (Dorfman et al., 2007; Edgell et al., 2007), as did exercises without concomitant LBNP during a 70-day HDBR study at the NASA FARU facility (see description of exercises in lean body mass and muscle section) (Scott et al., 2018). Rowing exercise (6 sets of 3-min high-intensity intervals) combined with upper- and lower-body resistance exercises also mitigated cardiac deconditioning (Hastings et al., 2012). Other benefits of these concurrent exercise countermeasures included preserved ventricular compliance (Hastings et al., 2012), and cardiac mechanics (LV strain and twist) (Scott et al., 2018). Similarly, resistive vibration exercise prevented changes in LVEDV, LV mass and contractility following 21-day HDBR (Greaves et al., 2019). Supine cycling alone (3 sessions x 30 min/day at 75% maximum HR) attenuated reductions to LVEDV and SV whilst maintaining cardiac mass and diastolic suction over 18 days of HDBR (Dorfman et al., 2008; Shibata et al., 2010), whilst supine cycling at only 40 W and combined with artificial gravity at alternating 1–2 Gz (foot level) preserved resting supine HR and systolic function during 4-day HDBR (Yang et al., 2011; Li et al., 2017). Overall, most HDBR studies provide evidence that aerobic and/or resistance exercise are beneficial for maintaining cardiac morphology and function, but differences in cardiac responses between concurrent and aerobic only countermeasures suggest the type and intensity of exercise is important for maintenance of cardiac function.

Older adults

Aging results in profound cardiac changes, even in healthy individuals. With natural increases in blood pressure, greater stress is put on the left ventricle to pump blood, leading to myocardial hypertrophy and fibrosis (Lakatta and Levy, 2003b; Strait and Lakatta, 2012; Ugander et al., 2012; Liu et al., 2013). This inevitably leads to impairment of contractile and relaxation properties of the left ventricle (Strait and Lakatta, 2012), while age-related reductions to maximal HR limits maximum $\dot{Q}$ (Lakatta and Levy, 2003b). Older adults also have higher risk of developing cardiac arrhythmias (Manolio et al., 1994), especially atrial fibrillation when atrial size is increased (Psaty et al., 1997). In a large study of enrolled adults up to 88 years of age, unfit individuals exhibited greater relative risk of cardiovascular-related deaths for both men (RR = 1.7) and women (RR = 2.42) (Blair et al., 1996). Furthermore, a recent meta-analysis confirmed the potent cardioprotective effects of aerobic training for maintenance of cardiac function in older men (>45 years) (Beaumont et al., 2019). Therefore, further investigation of exercise countermeasures during HDBR and extended periods of sedentary behaviour are essential.

Blood volume

Spaceflight

Early space research identified consistent losses to blood volume, with contraction of both plasma volume and red cell mass in-flight. During the Apollo program, astronauts landed with reductions in plasma volume of 4.4% and associated losses of 10% to red cell mass (Kimzey et al., 1975) This was subsequently followed by an immediate expansion of their intravascular volume by 4.8% after one day of recovery. Investigations of fluid balance during spaceflight are complicated by anecdotal reports of voluntary dehydration immediately prior to launch to prevent the need for urination, and by the possibility of space motion sickness with vomiting and reduced early fluid ingestion (Gharib and Hughson, 1992). Cephalad fluid shifts in microgravity and the subsequent negative fluid balance, fluid shifts between intravascular and interstitial spaces, and uncoupling of the kidney in space (Diedrich et al., 2007) (Table 1) all lead to a relative haemoconcentration during early spaceflight (Leach and Johnson, 1984). This uncoupling describes the lack of urinary fluid, sodium and urodilatin excretion on entry to microgravity despite elevated plasma atrial natriuretic peptide activity, as measured via urinary cyclic guanosine monophosphate excretion (Drummer et al., 2000). Although atrial natriuretic peptide activity is initially elevated, it subsequently falls whilst aldosterone and renin activity rise in-flight by day 100, a response to contraction of blood volume (Hughson et al., 2016). Despite evidence of reduced red cell production to correct an early haemoconcentration (Udden et al., 1995), later work suggests haemolysis of newly formed red blood cells (Alfrey et al., 1996) and/or persistent red cell haemolysis throughout flight can play an important role in haematological homeostasis (Trudel et al., 2022). Other than some evidence of pre-landing salt loading attenuating plasma volume losses (Leach et al., 1996), and the combination of exercise and salt loading preventing ambulatory orthostatic intolerance immediately post-flight (Fu et al., 2019), there is little evidence of the role of exercise alone in preventing blood losses during flight.

Bedrest studies

There are differences in the mechanisms through which blood volume is regulated in space when compared to ground studies (Table 1). Importantly, the increases in left atrial pressure and subsequent Gauer-Henry reflex (Gauer et al., 1961) are short-lived or non-existent during spaceflight, whilst persisting in analogue studies with a concomitant and significant diuresis (Hughson and Bondar, 1999; Diedrich et al., 2007). However, the plasma volume losses are similar, so analogue studies do provide important evidence for the role of exercise during periods of inactivity and immobility, with translational results for both terrestrial and extraterrestrial environments. Nevertheless, analogue studies cannot reproduce the unloading of the thorax or reduction in CVP experienced in microgravity, as CVP initially rises during HDBR but then falls back to baseline levels within 20 h (Gaffney et al., 1985). Therefore, HDBR results must be interpreted whilst considering that the physiology leading to blood loss is different between HDBR and spaceflight.

In young adults, exercise modality and intensity appear to be an important determinants of blood volume outcomes during HDBR. Daily aerobic supine cycling at 75% maximal HR (3 sessions x 30-min/day) prevented plasma volume losses following HDBR (Shibasaki et al., 2003; Shibata et al., 2010). Additionally, one bout of maximal supine cycle exercise to exhaustion during HDBR resulted in a complete reversal of HDBR-induced plasma volume losses over the subsequent 24 h (Convertino et al., 1996). Countermeasures during the WISE-2005 study also proved beneficial (Guinet et al., 2009). Conversely, other exercise modalities appear to have less of a protective effect on blood volume. Isotonic (68% of maximal $\dot{V}O_2$) and isometric exercise (21% maximal leg extension force) lasting half an hour each per day did not prevent plasma volume losses during two weeks of supine bedrest (Dolkas and Greenleaf, 1977). During HDBR, neither high-intensity jump training nor resistance vibration exercise showed any protective effects on blood volume (Kramer et al., 2017b; Guinet et al., 2020). The cross-over study design of Guinet et al. provides compelling evidence for the lack of benefit of lower-limb resistance exercise with concurrent vibration at 24Hz, as the exercise intervention failed to attenuate plasma losses of approximately 15% over 21 days of HDBR (Guinet et al., 2020). Given the noticeable differences in outcomes between studies, clarification of the type and intensity of exercises needed to maintain blood volume is an important step for adequate countermeasure activity in astronauts.

Older adults

Blood volume has been reported to decline with age (Gibson and Evans, 1937; Davy and Seals, 1994; Jones et al., 1997), but others have not found such an association (Carrick-Ranson et al., 2013). Regardless of age, physical activity is a critical determinant of blood volume (Convertino, 2007), and by maintaining regular physical activity older adults have comparable blood volumes to younger active adults (Jones et al., 1997). Given the importance of blood volume in maintaining blood pressure during orthostatic challenge (Shibata et al., 2010) and carrying oxygen to active muscles (Jones et al., 1997; Lundgren et al., 2021), establishing countermeasures to preserve blood volume during periods of bedrest is critical to preserve physical function in older adults following immobility.

Cerebrovascular function

Spaceflight

Limited data exist on the adaptation of the cerebral vasculature and cerebral autoregulation (CA) to spaceflight. During a 16-day Neuro-lab mission, static CA remained intact with enhanced dynamic CA post-flight (Iwasaki et al., 2007). Middle cerebral artery velocity (MCAv) was unchanged during short-duration spaceflight (Arbeille et al., 2001; Iwasaki et al., 2007). Although evidence suggests that those who exhibit orthostatic intolerance (OI) following spaceflight could not regulate cerebral blood flow (determined through MCAv) in response to decreased mean arterial pressure at the level of the brain (Blaber et al., 2011). Long-duration spaceflight appears to impair dynamic CA, although static CA remained intact (Zuj et al., 2012). Cerebrovascular CO_2 reactivity was also blunted, possibly as a consequence of the higher ambient CO_2 aboard the ISS (Zuj et al., 2012). Resting supine mean arterial pressure at the level of the brain was maintained after long-duration flight (Zuj et al., 2012; Iwasaki et al., 2021); however, resting MCAv remained stable (Zuj et al., 2012) or increased (Iwasaki et al., 2021) compared to pre-flight values. During brief microgravity exposures, directly measured intracranial pressure decreased (Lawley et al., 2017) and estimated intracranial pressure remained unchanged after six months in space (Iwasaki et al., 2021). During resistive exercise in microgravity intracranial pressure did transiently increase, likely due to a Valsalva (Lawley et al., 2017). However, this repeated transient increase of intracranial pressure during resistive exercise appeared to have no impact on long-term estimated intracranial pressure measured after spaceflight (Iwasaki et al., 2021).

Bedrest studies

The impact of exercise on cerebrovascular function during HDBR appears equivocal. Without exercise countermeasures, resting MCAv was increased (Kawai et al., 1993), maintained (Zhang et al., 1997; Arbeille et al., 2001; Pavy-Le Traon et al., 2002) or decreased (Frey et al., 1993; Arbeille et al., 1998) in HDBR studies ranging from 24 h to 42 days. Cerebrovascular resistance index (CVRi) was also increased during four-day HDBR (Arbeille et al., 1998), or maintained during seven (Pavy-Le Traon et al., 2002) and 42-day HDBR (Arbeille et al., 2001).

There does not appear to be any effect of resistance and/or aerobic exercise on end-tidal partial pressure of carbon dioxide ($P_{ET}CO_2$), MCAv, or CVRi during HDBR (Jeong et al., 2012, 2014; Kermorgant et al., 2019). The 60-day WISE-2005 study also identified no effect of exercise on cerebrovascular autoregulation, although all participants exhibited lower $P_{ET}CO_2$ with greater MCAv for any given $P_{ET}CO_2$ (Greaves et al., 2007). Aerobic cycling exercise had no impact (Jeong et al., 2012) or was detrimental to dynamic CA (Jeong et al., 2014), as was resistance vibration exercise (Kermorgant et al., 2019); other studies identified maintenance or even improvement of dynamic CA in sedentary control groups (Jeong et al., 2012; Kermorgant et al., 2019). Inter-individual variation appears to play a larger role in CA responses after HDBR rather than the exercise countermeasures themselves (Greaves et al., 2007). Overall, there is no clear effect of exercise on cerebrovascular function during HDBR.

Older adults

Cerebral blood flow decreases with age (Graff et al., 2022), as MCAv is reduced and cerebrovascular resistance is increased (Zhu et al., 2011). Inefficient CA can also occur with aging, but CA is quite variable within the population of healthy older adults (Liu et al., 2016; de Jong et al., 2017). Furthermore, arterial stiffening impairs the ability for the brain to buffer changes in cerebral blood flow pulsatility, risking the development of cerebral small vessel disease (Tarumi and Zhang, 2018) and subsequent cognitive impairment, gait disturbances, and even stroke (Li et al., 2018). "Life-long" and short-term aerobic exercise helps preserve cerebrovascular function, including helping maintain cerebral blood flow, compared to sedentary individuals of the same age (Chapman et al., 2013; Tarumi et al., 2013; Thomas et al., 2013). Indeed after just 10 days of no exercise, older endurance athletes had a significant reduction in cerebral blood flow (Alfini et al., 2016). As aerobic exercise has a clear benefit to cerebrovascular function in older adults, further assessments of aerobic and resistance exercises, and their benefits on cerebrovascular function during HDBR are required.

Vascular adaptions

Spaceflight

Recently identified vascular adaptations to spaceflight occurred despite access to newer exercise equipment and current exercise countermeasure regimes aboard the ISS. Reduced pulse wave transit times to the finger (Baevsky et al., 2007; Hughson et al., 2016) and ankle (Hughson et al., 2016) after prolonged spaceflight indicates increased central and peripheral artery stiffness. Changes in common carotid artery (CCA) distensibility coefficient and β-stiffness index after six-month missions also suggest arterial stiffening (Hughson et al., 2016; Arbeille et al., 2017), with the magnitude equivalent to 10–20 years of normal aging on Earth (Kawasaki et al., 1987; Gepner et al., 2014). Similar changes in CCA distensibility and β-stiffness were also reported by Lee et al. (2020), although their chosen statistical methodologies did not achieve significance. Men had greater changes in pulse wave transit time and women had greater changes in β-stiffness (Hughson et al., 2016), but larger sample sizes are needed confirm these findings. Assessment of ultrasound backscatter energy from astronauts' CCAs support a hypothesis of arterial wall remodeling with altered arterial wall content underlying observed changes in stiffness (Arbeille et al., 2021). Furthermore, insulin resistance and hyperglycemia were reported in astronauts after six months in space (Hughson et al., 2016), which could also contribute to changes in vascular properties (Webb et al., 2010).

Common carotid artery intima-media thickness (IMT), which is clinically used as a surrogate marker for atherosclerosis (Coll and Feinstein, 2008), increased by 12% over six months in space (Arbeille et al., 2016) and by ~20% in the one-year NASA twin study (Garrett-Bakelman et al., 2019); however, results have not been consistent (Lee et al., 2020). Most of the increase in CCA IMT (10%) was reported to occur within the first 15 days of spaceflight (Arbeille et al., 2016), with similar changes (+15%) in the superficial femoral artery (SFA) (Arbeille et al., 2016). Interestingly, increases in CCA IMT were observed during the Mars-500 confinement study (Arbeille et al., 2014), where men lived isolated, albeit upright in normal gravity, suggesting that non-gravitational factors can influence IMT measurements (Arbeille et al., 2016). Brachial (Lee et al., 2020) and SFA (Arbeille et al., 2016) diameters change during long-duration spaceflight, with mixed outcomes for the CCA diameter, as both increases 5%–7% (Garrett-Bakelman et al., 2019; Lee et al., 2020), or no change have been reported (Arbeille et al., 2016). Furthermore, endothelium-dependent flow-mediated dilation (FMD) and endothelium-independent nitroglycerin vasodilation of the brachial artery were unchanged following flight (Lee et al., 2020). In summary, despite current exercise countermeasures, astronauts' arteries become stiffer with increased IMT.

Bedrest studies

Bedrest and physical inactivity are strong stimuli for vascular remodelling. It is well established that exercise and its associated haemodynamic responses enhance vascular function and structure, but distinct local vascular adaptations and benefits of exercise depend on the its modality, duration, and intensity (Green et al., 2017). To date, no prolonged bedrest study has evaluated the effects of exercise countermeasures on arterial stiffness. However, the effect of HDBR without exercise over 60 days, appears equivocal. Möstl et al. (2021) found no change in brachial-femoral pulse wave velocity, aortic distensibility, or corrected pulse arrival time for isovolumetric contraction time to different vascular beds (arm, finger, thigh, and toe). Furthermore, they stratified their sample into age tertiles, finding no evidence of increased risk for vascular changes in their older (37–55 years) compared to younger participants (24–36 years), despite higher brachial-femoral pulse wave velocity with greater age (Möstl et al., 2021). In contrast, Fayol et al. (2019) reported increased carotid-femoral pulse wave velocity during 60 days of HDBR, which remained elevated even after one year of recovery. HDBR of 35 days also did not result in changes to carotid-femoral pulse wave velocity (Palombo et al., 2015). Given the disparate responses between studies (Palombo et al., 2015; Fayol et al., 2019; Möstl et al., 2021), clarification of prolonged bedrest effects on central artery stiffness and the potential benefits of exercise are required. If eventually concluded that bed rest and spaceflight do not lead to similar changes in arterial stiffness, it may suggest that other factors, such as space radiation exposure, changes in blood glucose, or altered hormonal background (Hughson et al., 2018), are important factors in the vascular changes that occur during long-duration spaceflight.

Vascular remodelling occurs with the absence of exercise during supine or HDBR, leading to reduced diameters of arteries feeding locomotor muscle groups, including the common femoral (Bleeker et al., 2005; Palombo et al., 2015), SFA (Bleeker et al., 2005) and popliteal arteries (Dyson et al., 2007), but CCA diameter appears unaffected by bedrest (Bleeker et al., 2005; van Duijnhoven et al., 2010b; Palombo et al., 2015). Vascular diameter remodeling occurs rapidly and follows a non-linear time course, as during the first Berlin bedrest study, common femoral artery diameters reduced by ~13% over the first 25 days of a 52-day supine bedrest campaign, and only fell by ~4% more in the final 27 days (Bleeker et al., 2005). Lower-body resistive exercises were able to attenuate these decreases by ~5% and ~6% at days 25 and 52, respectively (Bleeker et al., 2005). Importantly, these exercises only provided locally protective effects, as brachial artery diameters fell in both control and exercisers alike (Bleeker et al., 2005). Subsequently, the second Berlin bedrest study revisited these findings, comparing lower-body resistive exercise with or without vibration. Exercises included squats (10 reps, 80% maximum force, ±20–24 Hz vibration), single-leg heel raises (to exhaustion, 1.3× body weight, ±26 Hz), double-leg heel raises (to exhaustion, 1.8× body weight, ±24 Hz) and back and heel raises (full extension for 60 s, 1.5× body weight, ±16 Hz) for three days every week. Again, resistance exercise attenuated reductions to SFA diameter, with greater attenuation in the vibration group (van Duijnhoven et al., 2010b). Results from the WISE-2005 study corroborated the protective effects of exercise on arterial remodelling (Dyson et al., 2007).

Following 35 days of HDBR without countermeasures, no change in CCA IMT was noted (Palombo et al., 2015). In the second Berlin bedrest study, thickening of the walls of the CCA (+17% IMT) and SFA (+13% IMT) were induced by 60 days of HDBR, whilst exercises with and without vibration helped prevent these increases (van Duijnhoven et al., 2010a). Given the magnitude of change in IMT in the control group over a short period of time (~75 times greater than would be expected with 60 days aging), and the protective effects of exercise, IMT changes appear sensitive to activity level and do not indicate rapid development of atherosclerosis in these particular circumstances (van Duijnhoven et al., 2010a).

FMD responses from bedrest studies indicate paradoxical results (i.e., enhanced vasodilation suggesting improved endothelial function following prolonged inactivity), as responses in the leg were greater following 49-day HDBR (Platts et al., 2009). Exercise countermeasures during the WISE-2005 bedrest campaign (Dyson et al., 2007), and during the Berlin bedrest studies (Bleeker et al., 2005; van Duijnhoven et al., 2010b) were able to maintain or dampen increases in popliteal and SFA FMD responses after prolonged bedrest. The inverse relationship between resting artery diameter and proportional dilation (van Duijnhoven et al., 2010b) suggests that as arteries become smaller due to inactivity during bedrest, they dilate more. Therefore, in order to correctly interpret FMD findings during bedrest studies, an understanding of diameter-percent FMD relationships is critical (Thijssen et al., 2008).

The effect of bedrest on arterial stiffness remains unclear; however, regional artery dimensions are sensitive to inactivity. Exercise countermeasures have a critical role in maintaining vessel diameter but have focused primarily on lower-body exercise. Moving forward, multi-modal, whole-body exercise countermeasures that utilize both the upper and lower body should be explored to ensure that vascular protection is not localized to only certain branches of the vascular tree.

Older adults

Older age is associated with increases to IMT (Karikkineth et al., 2020) and endothelial dysfunction (Seals et al., 2011). Additionally, arteries stiffen due to (amongst others) increased calcification, collagen production, and inflammation, resulting in increases to pulse-wave velocity and pulse pressure within vessels (Donato et al., 2018). This increases the risk of developing hypertension and cardiovascular disease (Mitchell et al., 2010). In sedentary but otherwise healthy older adults, both continuous aerobic training and resistance training multiple days a week for 8–12 weeks reduced arterial stiffness, but high-intensity interval training did not (Kim et al., 2017; Park et al., 2020; Akazawa et al., 2021). Notably, two weeks of bedrest in older adults impaired endothelial function (Goswami et al., 2015), but changes in other arterial properties of healthy older adults during bedrest, and the potential benefit of concomitant exercise remain unexplored.

Autonomic changes

Spaceflight

Autonomic control of the cardiovascular system is altered by spaceflight, and its function is critical for regulation of blood pressure, especially during postural changes. Following short-duration flights, vagal baroreflex responses were impaired (Fritsch et al., 1992; Fritsch-Yelle et al., 1994; Eckberg et al., 2010), and increases in peripheral resistance and norepinephrine were attenuated upon standing (Fritsch-Yelle et al., 1996). In-flight measures of vagal baroreflex sensitivity during the Neurolab mission were also reduced (Cox et al., 2002), and muscle sympathetic nerve activity (MSNA) and norepinephrine spillover were elevated in-flight and post-flight, with enhanced sympathetic responses observed to LBNP in space (Ertl et al., 2002). These findings suggest enhanced sympathetic activation in space and during the days following return to Earth. Importantly, the sympathetic reflex responses to orthostatic challenge following short-duration spaceflight (Levine et al., 2002; Beckers et al., 2009), and hand-grip and cold-pressor tests performed in-flight and post-flight (Fu et al., 2002) were appropriate in magnitude, which indicated that sympathetic mechanisms of cardiovascular control remained intact. Trends towards increased sympathetic activity during spaceflight were identified by the ratio of low-to-high frequency power from HR variability analysis, but are difficult to interpret due to postural and respiratory effects (Mandsager et al., 2015). Vagal baroreflex sensitivity was maintained during long-duration missions on the ISS in-flight, but reduced immediately upon landing (Hughson et al., 2012), albeit less than expected based on previous short-duration spaceflight findings (Fritsch et al., 1992; Fritsch-Yelle et al., 1994) and following 9 months on the Mir space station (Cooke et al., 2000). This decreased vagal activity abates quickly and may be appropriate for the stress of standing in astronauts immediately following landing (Beckers et al., 2009). Autonomic control of astronauts' heart rate following both short- and long-duration spaceflight appeared to recover to pre-flight levels within 30 days of returning to Earth (Vandeput et al., 2013). Therefore, current in-flight exercise countermeasures help maintain cardiovascular control (Hughson et al., 2012).

Bedrest studies

HDBR reduced parasympathetic nervous system activity (Hughson et al., 1994b; Crandall et al., 1994; Sigaudo et al., 1998), with mixed effects on the sympathetic nervous system. HR variability analysis suggests increased (Hughson et al., 1994b; Coupé et al., 2011) or unchanged sympathetic activity (Crandall et al., 1994; Sigaudo et al., 1998), while MSNA post-HDBR was increased (Kamiya et al., 2000; Barbic et al., 2019), unchanged (Pawelczyk et al., 2001) or decreased (Shoemaker et al., 1998). Interestingly, some individuals who presented with OI post-bedrest, had attenuated increases in MSNA (Kamiya et al., 2003), while others had normal MSNA responses to orthostatic stress, but reduced vasomotor response to sympathetic outflow (Arbeille et al., 2008). Similar to spaceflight, HDBR reduced vagal baroreflex response (Convertino et al., 1990; Hughson et al., 1994a; Sigaudo et al., 1998; Iwasaki et al., 2004). The reduction in plasma volume with bedrest has been implicated in the change of baroreflex sensitivity, as plasma volume restoration normalizes the spontaneous baroreflex function (Iwasaki et al., 2004). However, others reported that the change in baroreflex slope did not occur in parallel with changes to blood volume and persisted throughout the first week of recovery (Convertino et al., 1990), which suggests effects of bedrest on autonomic control independent of fluid status.

There are limited data available regarding the effectiveness of consistent exercise interventions alone to prevent the autonomic changes during and after HDBR. A single bout of dynamic and isometric high-intensity leg exercise, which leads to baroreceptor loading due to elevations in blood pressure, appeared to temporarily reverse reductions in vagal-mediated baroreflex function for ~24 h following seven days of HDBR (Convertino et al., 1992). Other high-intensity resistance exercise interventions, such as reactive jump training (Maggioni et al., 2018) or resistance exercise combined with vibration (Coupé et al., 2011), appeared to help maintain resting sympathovagal balance following 60 days of HDBR. Resistance exercise combined with vibration slightly attenuated the reduction in baroreflex sensitivity following 60 days of HDBR (Coupé et al., 2011). Alternatively, the combination of aerobic exercise and artificial gravity was very effective at attenuating changes parasympathetic activity and baroreflex function post-bedrest (Iwasaki et al., 2005); however, delaying the start of artificial gravity and high-intensity exercise countermeasures by seven days, or possibly insufficient time dedicated to the countermeasure, reduced its effectiveness (Hughson et al., 1994a).

Overall, changes in autonomic cardiovascular control during and following HDBR involves many complex interactions between different parts of the cardiovascular system, leading to variable responses. Exercise alone transiently improves indices of autonomic function and can provide some benefit for maintaining baroreflex sensitivity and sympathovagal balance.

Older adults

Autonomic function tends to decline with age, as sympathetic activity increases (Seals and Esler, 2000), and vagal baroreflex sensitivity is reduced (Ebert et al., 1992). Research indicates that aerobic exercise training improves cardiac autonomic control in older adults, determined by heart rate variability (Grässler et al., 2021), as well as baroreflex sensitivity (Madden et al., 2010). High-intensity interval training has been reported to confer similar autonomic benefits in older adults (Pichot et al., 2005), although others have not found an effect (Cassidy et al., 2019). Accordingly, it is unknown if exercise countermeasures are also effective at maintaining autonomic function in older adults during periods of immobility.

Glucose handling

Spaceflight

Early spaceflight indicated potential alterations to glucose handling over the course of a 150-days Salyut 7-Soyuz T9 sojourn, with delayed time to peak blood glucose during oral glucose tolerance test (OGTT) by flight day 60 through to post-flight day 25 in one cosmonaut (Alexandrov et al., 1985). OGTT responses in one astronaut whilst aboard the Mir space station also indicated greater plasma insulin and glucose concentrations compared to pre-flight responses (Macho et al., 2003). Later results from shuttle flights supported these findings, with measurements of c-peptide suggestive of increased insulin secretion to overcome in-flight insulin resistance (Stein et al., 1994), although these data were later combined with further flight data that questioned this initial finding (Stein et al., 1999b). The Spacelab D2 mission (1993) included OGTT assessment of four astronauts, finding higher mean glucose, insulin, and c-peptide concentrations in-flight, despite them remaining within normal ranges (Maaß et al., 1997). It was not until recently that statistically significant increases in insulin resistance index and close-to-significant increases in glycated albumin were confirmed in both male and female astronauts aboard the ISS (Hughson et al., 2016). Importantly, these data identified altered glucose handling in astronauts performing the most up-to-date exercise countermeasure activities. However, consideration of astronauts' absolute exercise duration must be made (Fraser et al., 2012; Hughson et al., 2016). Thus, the current duration, intensity, and/or modality of exercise during spaceflight appears to be insufficient to prevent altered glucose handing given the limited data we have from space.

Bedrest studies

HDBR is known to cause impaired insulin sensitivity as documented in several different studies (Blanc et al., 2000; Heer et al., 2014; Montero et al., 2018; Rudwill et al., 2018). However, only a small number of investigations studying the beneficial effects of exercise on insulin resistance exist. Isotonic and isometric exercises reduced insulin and glucose responses to OGTT compared to control subjects in a three-arm cross-over supine bedrest and were inversely proportional to energy expenditure during each campaign (Dolkas and Greenleaf, 1977). During long-duration HDBR of 60 and 70 days, both resistive vibration exercise (Yang et al., 2014) and a combination of resistive and aerobic exercise (Downs et al., 2020) attenuated the detrimental effects on insulin tolerance. Potential mechanisms include transmembrane GLUT-4 content in skeletal muscle, as maximal isometric leg-press exercises increased concentrations in VL during 19-day HDBR, whilst control participants experienced relative decreases (Tabata et al., 1999). However, ongoing research into the potentially protective role of exercise during periods of HDBR is needed.

Older adults

Incidence of insulin resistance increases with age, with reasons including reduced physical activity (Bauman et al., 2016; McGlory et al., 2018), and intrinsic changes related to skeletal muscle aging (Shou et al., 2020). However, consistent physical activity with little sedentary time appears to improve glucose metabolism in older adults (Länsitie et al., 2021). Both endurance and resistance training improve glucose metabolism in older adults, especially in those who were previously sedentary (Consitt et al., 2019). High-intensity interval training can also enhance glycemic control (Cassidy et al., 2016). Even as little as 2000 steps/day in older adults undergoing seven days of bedrest preserved glucose metabolism in older adults (Arentson-Lantz et al., 2019). Considering this, glucose handling assessment in older populations during HDBR with exercise countermeasure interventions may provide clinically important information that could inform medical practice.

Orthostatic intolerance

Orthostatic intolerance occurs when delivery of oxygen to the brain in upright posture is insufficient resulting in symptomatology, including light-headedness, dizziness, and even syncope (Van Lieshout et al., 2003). Spaceflight and bedrest both induce cardiovascular changes that hinder the supply of oxygen to the brain during orthostatic stress. Mechanisms contributing to OI after spaceflight or bedrest are multifaceted, including failure to maintain arterial blood pressure due to changes in cardiac function coupled with reduced blood volume and venous return (Levine et al., 1997; Perhonen et al., 2001b; Shibata et al., 2010) or inadequate arterial vasoconstriction (Buckey et al., 1996b; Waters et al., 2002), as well as impaired CA (Zhang et al., 1997). However, OI has been identified in cases with maintained arterial blood pressure but paradoxical cerebral vasoconstriction (Grubb, 2005; Novak, 2016).

Spaceflight

OI was first noted during Mercury missions, with symptom severity dependent on mission duration (Link, 1965). OI continued to be reported following longer 14-day Gemini flights (Berry et al., 1966), where noticeable inter-individual variability was reported (Berry and Catterson, 1967). Even with the introduction of routine onboard exercise countermeasures, OI persists, with increasing prevalence at landing as mission length increases (Meck et al., 2001). Despite access to the iRED and TVIS devices, ISS astronauts who flew for ~177 days had far greater rates of OI compared to shuttle astronauts flying for ~17 days (Lee et al., 2015). Additionally, recent studies of eight long-duration astronauts identified two that experienced orthostatic hypotension during a three-minute stand test (Wood et al., 2019). As formal orthostatic testing equates to motionless standing without muscle contraction, Fu et al. (2019) assessed OI during activities of daily living in 12 astronauts following approximately six months in space (between 2009–2013) finding no evidence of OI or orthostatic hypotension. Therefore, standing still following landing should be discouraged in returning astronauts, and activity encouraged as a countermeasure. Another possible way to reduce risk of OI is *via* fluid loading immediately prior to landing (Bungo et al., 1985). During shuttle missions, approximately one-half of the astronauts used such a protocol, but risk of OI was unaffected (Buckey et al., 1996b). Today, flight surgeons recommend fluid loading prior to landing, but there are no systematic data to evaluate the efficacy of this countermeasure. With regards to identifying "at-risk" individuals, tolerance to upright posture following landing has great inter-individual variability (Buckey et al., 1996b; Levine et al., 2002; Meck et al., 2004; Blaber et al., 2011). Women are more likely to experience OI after spaceflight, possibly related to differences in CA, or lower peripheral vascular resistance and hypo-adrenergic responses (Fritsch-Yelle et al., 1996; Waters et al., 2002; Blaber et al., 2011), but prediction otherwise remains difficult.

Bedrest studies

Without countermeasures, prevalence of OI during orthostatic testing increased after as little as four-day HDBR (Arbeille et al., 1998). Similar results were observed following seven (Custaud et al., 2002), 14 (Zhang et al., 1997; Grenon et al., 2004), 21 (Barbic et al., 2019), 42 (Pavy-Le Traon et al., 1998), and 60 days (Liu et al., 2015). Unlike spaceflight, rates of OI were similar between men and women (Custaud et al., 2002), although only a few studies have included both sexes in the experimental design (Watenpaugh et al., 2007).

Exercise alone during HDBR does not appear to provide complete protection from OI. Varied aerobic rowing and resistance exercise 6-days/week during 35-day HDBR (Hastings et al., 2012) or 90-minutes of daily aerobic cycling at 75% maximum HR during 18-day HDBR (Shibata et al., 2010; Jeong et al., 2012) both failed to preserve orthostatic tolerance. However, when combined with plasma volume restoration, orthostatic tolerance was preserved (Shibata et al., 2010; Hastings et al., 2012; Jeong et al., 2012). Surprisingly, the aforementioned aerobic cycling actually exaggerated OI following HDBR compared to controls (Shibata et al., 2010; Jeong et al., 2012). Resistance vibration exercise was not found to have any impact on reducing OI compared to a sedentary control group after 21 days (Guinet et al., 2020) and 90 days of HDBR (Belin de Chantemèle et al., 2004). The introduction of a gravitational stimulus using supine treadmill exercise inside LBNP attenuated the reduction (-13%) in orthostatic tolerance time compared to controls (−34%) following 30 days of bedrest (Watenpaugh et al., 2007). The WISE-2005 study utilized similar exercise countermeasures but appeared less effective, as orthostatic tolerance time was reduced by 35% in the countermeasure group compared to 50% in controls, although a lack of exercise in the final 62 h may have confounded these results (Guinet et al., 2009). Overall, the ability of exercise to attenuate OI after HDBR is complex and dependent on synergistic effects with other simultaneous countermeasures. Furthermore, individual physical and physiological factors are likely playing a role in OI susceptibility (Pavy-Le Traon et al., 1999), making it difficult to draw concrete conclusions.

Older adults

In adults over the age of 60, one in five experience OI, which increases to approximately one in four if residing in long-term care (Saedon et al., 2020). Presence of OI is associated with increased risk of falls (Finucane et al., 2017), cardiovascular and cerebrovascular morbidities (Ricci et al., 2015), and frailty (O'Connell et al., 2015). Mild to moderate exercise is often encouraged as part of an OI management plan (Figueroa et al., 2010), as OI is exacerbated by inactivity. With the low cost and ease in prescribing potentially effective exercise countermeasures, assessment of the efficacy of exercise in older adults is important, especially during periods of protracted immobility.

Aerobic fitness

The aerobic response to exercise provides important information about the integrative physiological effects of microgravity and bedrest on multiple physiological systems. The marked cardiovascular and musculoskeletal changes during spaceflight and inactivity alter the oxygen transport cascade and oxygen utilization. Reduced blood volume limits potential increases to $\dot{Q}$ due to impaired cardiac filling, reduced SV and elevated resting HR (Convertino, 1997), while decreased conduit artery diameters further limit oxygen delivery to exercising muscles (Dinenno et al., 2001; Miyachi et al., 2001). Loss of muscle mass and myofiber shifts to less oxidative types impair aerobic metabolism (Trappe et al., 2009). Even orthostasis (upright vs. supine) can impair the supply of oxygen to working muscles post-bedrest (Convertino et al., 1982; Hung et al., 1983). A simple integrative outcome assessing these whole-body physiological consequences and the potential benefit of exercise is maximal rate of oxygen uptake ($\dot{V}O_2$max).

Spaceflight

It was identified early in human spaceflight that exercise countermeasures were needed to attenuate deconditioning and preserve work ability [see review by Moore et al. (Moore et al., 2010)]. Limited $\dot{V}O_2$max data were collected during early missions, but submaximal $\dot{V}O_2$ and HR data were suitable alternatives. Following short-duration Gemini and Apollo missions, reductions in aerobic fitness were inferred from large reductions in $\dot{V}O_2$ (19%–26%) and work rate for a given HR post-flight (Dietlein and Rapp, 1966; Berry and Catterson, 1967; Rummel et al., 1973). This prompted the implementation of more extensive exercise countermeasures (see Figure 2) with allocated daily exercise of 30–90 min during the Skylab missions (Johnston, 1977). Increasing exercise intensity and duration better maintained astronauts' fitness in-flight (Michel et al., 1975, 1977; Rummel et al., 1976) and post-flight (Greenisen et al., 1999), while limited exercise during shuttle missions due to time constraints resulted in decreases to post-flight fitness (Levine et al., 1996).

During six-month missions to the ISS, average $\dot{V}O_2$max (−17%) and peak work rate (−24%) fell early in-flight, gradually recovering but never reaching pre-flight levels (Moore et al., 2014). Immediately post-flight, $\dot{V}O_2$max was reduced (−15%), returning to pre-flight levels within 30 days of landing (Moore et al., 2014; Lee et al., 2020). However, there was large variation in the effectiveness of the exercise countermeasures between astronauts; despite reductions to average $\dot{V}O_2$max, half of those studied achieved in-flight values at or above pre-flight levels, with a few subsequently experiencing only minimal reductions at landing (Moore et al., 2014). Fitter individuals had the greatest reduction in $\dot{V}O_2$max, while those performing high-intensity exercise training (>80% maximum HR on CEVIS and treadmill), or those with relatively lower pre-flight fitness maintained fitness during flight (Moore et al., 2014). High intensity-low volume training appears to confer similar benefits for aerobic fitness (English et al., 2020). Therefore, spaceflight-induced reductions in aerobic fitness can be eliminated if exercise countermeasures are properly prescribed.

Bedrest studies

Aerobic fitness appears to decay during bedrest relatively linearly, as revealed in a recent meta-analysis [0.43% (−0.22 ml/kg/min) per day] (Ried-Larsen et al., 2017), although the rate of decay might be faster in the first 10 days (Capelli et al., 2006; Ade et al., 2015). As with spaceflight, individuals with higher pre-bedrest $\dot{V}O_2$max lose more when compared to lower fitness individuals (Ried-Larsen et al., 2017). Following shorter duration bedrest studies (up-to two weeks), individuals typically fully recover fitness within one week (Trappe et al., 2006), with slower recovery following longer duration bedrest (Sundblad et al., 2000).

Various exercise countermeasures implemented during bedrest studies attempted to preserve aerobic fitness. Low-intensity aerobic exercise (40% $\dot{V}O_2$max) up to one hour daily was insufficient to prevent loss of aerobic fitness (Suzuki et al., 1994), whilst an hour of daily moderate-intensity (68% of $\dot{V}O_2$max) exercise partially attenuated reductions in $\dot{V}O_2$max (Stremel et al., 1976). Therefore, higher intensity or larger training volumes are required to maintain aerobic fitness during bedrest. Ninety minutes of daily cycling exercise (75% maximum heart rate) prevented losses to $\dot{V}O_2$max after 18 days of bedrest (Shibasaki et al., 2003), while two 30-min high-intensity interval training sessions performed five days per week maintained $\dot{V}O_2$max over a 30-day bedrest campaign (Greenleaf et al., 1989). Daily aerobic exercise coupled with volume loading at the end of bedrest to restore plasma volume maintained $\dot{V}O_2$max (Shibata et al., 2010). Resistance exercise attenuated reductions in $\dot{V}O_2$max (Stremel et al., 1976), and a combination of lower-body resistance exercise and whole-body vibration prevented significant reductions in $\dot{V}O_2$max (Guinet et al., 2020). One hour or less of total daily exercise, consisting of high-intensity interval training in combination with continuous aerobic and resistance training, maintained or even improved $\dot{V}O_2$max following 14 days of bed rest (Ploutz-Snyder et al., 2014). Another effective high-intensity, low-volume exercise type is sledge jumping, as 48 exercise sessions (3 min of exercise per session) spread over 60 days of HDBR prevented reductions in aerobic fitness (Kramer et al., 2017a). More recently, hybrid countermeasures combining both exercise and artificial gravity garnered more research focus, as exercise and artificial gravity appear to work synergistically to maintain fitness and attenuate cardiovascular changes. The use of centrifugation (Katayama et al., 2004) or LBNP (Watenpaugh et al., 2000; Lee et al., 2007) with simultaneous high-intensity interval exercise has been very effective at maintaining aerobic fitness post-bedrest. Moderate-intensity cycling during artificial gravity also maintained $\dot{V}O_2$max during four-day HDBR (Li et al., 2017). The addition of resistance training to aerobic training with LBNP also maintained aerobic fitness post-HDBR (Schneider et al., 2009). However, artificial gravity in the absence of exercise was not able to attenuate the bedrest associated reductions in $\dot{V}O_2$max (Kramer et al., 2021).

Given the relatively limited access to exercise equipment capable of simultaneous application of artificial gravity, both in space and on Earth, high-intensity interval exercise in combination with resistance training currently appears to be the most feasible countermeasure to attenuate loss of aerobic fitness.

Older adults

Older adults generally have lower fitness than younger adults (Betik and Hepple, 2008), but still experience larger reductions in aerobic fitness (12%–15%) after relatively short-duration (10–14 days) bedrest (Kortebein et al., 2008; Pišot et al., 2016). Physical activity levels tend to decline in older age (Hansen et al., 2019), but even those who remain as physically active as their younger counterparts exhibit lower average aerobic fitness (Aspenes et al., 2011). Immobility or bedrest can exacerbate losses in $\dot{V}O_2$max, leading to a decline in physical function (Coker et al., 2015). Aerobic and resistance exercises are strongly recommended for older adults in order to maximize aerobic fitness and reduce frailty (Izquierdo et al., 2021). Therefore, it is important to assess exercise regimes consisting of aerobic and resistance components and their ability to attenuate the loss in aerobic fitness in older adults during bedrest.

Considerations for the upcoming bedrest study in older adults

Older adult bedrest studies

To date, very few bedrest investigations in older adults have been undertaken, with most focussing on musculoskeletal consequences of bedrest without the inclusion of exercise countermeasures (Di Girolamo et al., 2021). Furthermore, the supine bed rest modality utilized in these studies does not invoke the same cardiovascular responses as those induced by 6° HDBR; indeed, HDBR in older adults is as-of-yet under-investigated.

Following supine bedrest, older adults show greater magnitudes of lower-limb muscle mass loss compared to younger adults, with reductions in muscle power that correlated with bedrest duration (Di Girolamo et al., 2021). After only 10-day bedrest, older adults already showed functional losses that include reduced stair-climbing and floor transfer times (Ferrando et al., 2010). Older adults also experienced greater proportional reductions in $\dot{V}O_2$max compared to younger adults for the same duration of bedrest (Pišot et al., 2016). Bedrest-induced cardiovascular changes in older adults are underexplored, although orthostatic intolerance (Pišot et al., 2016) and reduced endothelial function (Goswami et al., 2015) were reported following two-week supine bedrest. Magnitudes of other cardiovascular changes are relatively unknown, and must be evaluated given the age-related increases to rate of arterial stiffening with aging (Gepner et al., 2014) and greater cardiovascular disease risk (Lakatta and Levy, 2003a; 2003b). The detrimental and understudied physiological consequences of prolonged bedrest in older adults underscores the need for further identification and evaluation of appropriate exercise countermeasures to mitigate or prevent potentially pathological changes in this population.

Goals for the bedrest study in older adults study

As evidenced by the preceding review, exercise can help mitigate or completely prevent many musculoskeletal and cardiovascular consequences of short- and long-term spaceflight and bedrest. However, the majority of bedrest and HDBR studies have been conducted in a young population (Ried-Larsen et al., 2017) and older adult bedrest studies have not included exercise countermeasures without participants leaving their beds (Di Girolamo et al., 2021). Furthermore, a suite of countermeasures must be assessed, as any one modality appears insufficient to alleviate detrimental consequences in all physiological systems. It is important to explore exercise countermeasures in older adult bedrest studies for their clinical relevance as well as observations of astronauts older than 50 years of age participating in critical space missions (Kovacs and Shadden, 2017) and future space tourists. To date, there are not systematic data comparing younger and older astronauts. Debatably, the older population is most affected by long-term sedentary bedrest, such as during hospital stays, which can reduce physical fitness leading to a greater risk of adverse outcomes (Kortebein et al., 2008; Venturelli et al., 2012). Therefore, an important research question is apparent: does exercise reduce the negative musculoskeletal and cardiovascular impacts of HDBR in older adults? In a clinical trial funded by the Canadian Institutes for Health Research, the Canadian Frailty

TABLE 2 Proposed exercise countermeasure protocol for the bedrest study in older adults.

Week #1	Day 1	Day 2	Day 3	Day4	Day 5	Day 6	Day 7
Session 1	Resistance, Upper	Cont. Aerobic (30)	Progressive Aerobic	Cont. Aerobic (30)	HIIT	Cont. Aerobic (30)	HIIT
Session 2	Progressive Aerobic	Resistance, Lower	Cont. Aerobic (15)	Resistance, Upper	Progressive Aerobic	Resistance, Upper	Cont. Aerobic (15)
Session 3	HIIT	Progressive Aerobic	HIIT	Progressive Aerobic	Resistance, Lower	Progressive Aerobic	Progressive Aerobic
Week #2	**Day 8**	**Day 9**	**Day 10**	**Day 11**	**Day 12**	**Day 13**	**Day 14**
Session 1	Cont. Aerobic (15)	HIIT	Cont. Aerobic (30)	HIIT	Cont. Aerobic (30)	HIIT	Cont. Aerobic (15)
Session 2	Resistance, Upper	Cont. Aerobic (15)	Resistance, Lower	Cont. Aerobic (15)	Resistance, Lower	Progressive Aerobic	Cont. Aerobic (30)
Session 3	Progressive Aerobic	Progressive Aerobic	Progressive Aerobic	Progressive Aerobic	Progressive Aerobic	Resistance, Upper	Progressive Aerobic

Exercise type	Exercise time (min)	Equipment	Exercise description
HIIT	32	Cycle ergometer	5 min warm-up (40% HRR), 11 intervals (30s on @ 80%–90% HRR, 1.5 min relaxed cycling), 5 min cool-down
Cont. Aerobic (15)	15	Cycle ergometer	3 min warm-up (40% HRR), 9 min steady-state (60%–70% HRR), 3min cool-down (40% HRR)
Cont. Aerobic (30)	30	Cycle ergometer	5 min warm-up (40%–50% HRR), 20 min steady-state (60%–70% HRR), 5 min cooldown (40%–50% HRR)
Progressive Aerobic	15	Cycle ergometer	3 min stages at: 30%, 40%, 50%, 60%, 40% HRR
Resistance, Lower	25	Cables, resistance bands, body weight	3 sets (1 warm-up) of 10–12 repetitions
			Exercises: Hip raise, leg press, ankle pump, leg curls
Resistance, Upper	25	Cables, resistance bands, body weight	3 sets (1 warm-up) of 10–12 repetitions
			Exercises: External shoulder rotation, chest fly, lateral pull-down, dead bug

HRR, heart rate reserve.

Network, and Canadian Space Agency (NCT04964999), we propose assessing the effectiveness of a combination of high-intensity interval training (HIIT), continuous aerobic exercise, and upper- and lower-body resistance exercise to reduce musculoskeletal and cardiovascular deconditioning over 14 days of HDBR in older adults aged 55–65 years. The large multidisciplinary research team participating in the BROA study comprises experts in musculoskeletal and cardiovascular physiology, in addition to nutritional, cognitive, and medical specialists, and includes a wide range of hypotheses and investigative outcomes. Over the course of the following months and years, publications of exercise intervention efficacy in multiple physiological systems will further our understanding of potential countermeasure and rehabilitation practices aiding both terrestrial and extra-terrestrial individuals.

Exercise countermeasure protocol rationale

Twenty to 24 participants will be randomly assigned to one of two groups, sedentary control, or exercise. The sedentary control group will participate in daily physiotherapy stretching sessions, whereas the exercise group will participate in three daily training sessions all while maintaining six-degree head-down tilt (Table 2). The countermeasure program was designed to address several key physiological systems affected by bedrest or spaceflight. Three exercise sessions per day totalling one hour with four hours between sessions were included, as breaking up sedentary time is beneficial to cardiometabolic health (Healy et al., 2008; Bergouignan et al., 2016). HIIT was added to the protocol for several reasons. High-intensity training has beneficial effects on maintaining or improving aerobic fitness. HIIT was demonstrated by Cassidy et al. (Cassidy et al., 2016) to induce robust metabolic and cardiovascular benefits in clinical populations with type 2 diabetes. As well, inclusion of high-intensity exercise might achieve the benefits on baroreflex response reported by Convertino (Convertino et al., 1992), although HIIT per se has had mixed effects showing benefit (Pichot et al., 2005) and no effect (Cassidy et al., 2019). Overall, the cycling exercises and upper- and lower-body resistance protocols were chosen to resemble high-intensity, low-volume programs which have found some success in maintaining musculoskeletal and cardiovascular outcomes during spaceflight and HDBR in a younger population (Ploutz-Snyder et al., 2014, 2018; English et al., 2020). Although not discussed within the scope of this review, nutritional intake will be controlled according to the Guidelines for Standardization of Bedrest Studies in the Spaceflight Context in order to help maintain body weight (Angerer et al., 2014).

Conclusion

Spaceflight and HDBR without the use of exercise countermeasures cause loss of muscle mass and bone density, and marked deconditioning of the integrative function of the cardiovascular system, resulting in orthostatic intolerance and reduced aerobic fitness. Spaceflight studies conducted on middle-aged adults and HDBR studies focusing on young healthy adults demonstrated that a combination of aerobic and resistance exercise is best at maintaining or even improving musculoskeletal and cardiovascular systems variables. Exercise countermeasures during spaceflight or HDBR have beneficial effects on muscle mass and strength, muscle protein synthesis, cardiac morphology, and function, and vascular properties, leading to better aerobic fitness and orthostatic tolerance. However, the impact of exercise during spaceflight or HDBR on blood volume, cerebrovascular function, and autonomic function appears equivocal and should be the focus of future work. Furthermore, as we explain in this review, any one modality of exercise fails to attenuate all negative effects of spaceflight or HDBR. Indeed, despite the reported beneficial effects of specific exercise on isolated muscle groups or individual physiological systems, whole-body benefits appear limited for many of these countermeasure modalities. The need to formulate comprehensive and efficacious countermeasure regimes is paramount to ensure a holistic approach for both astronauts and immobile individuals throughout society. Finally, the current literature fails to address the effectiveness of exercise countermeasures during HDBR in older adults, even though they are more likely to be bed ridden, for example, during hospitalization. The bedrest-related decline in multiple physiological systems is exacerbated by age, necessitating assessment of exercise countermeasure interventions aimed at mitigating these changes. Therefore, we propose the BROA study, in which healthy older men and women, ages 55–65 years, will complete a 14-day HDBR campaign, with half the participants performing daily exercise during HDBR consisting of HIIT, aerobic, and resistance training. We hope the results from the BROA study will inform healthcare decisions that are especially important with transitions in care in older adults (Canadian Institutes of Health Research, 2021), and improve physiological outcomes on Earth and in space.

Author contributions

All authors contributed to drafting, editing, and revising the manuscript. All authors approved the final 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.

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Effects of space flight on sperm function and integrity: A systematic review

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With the advancement in space exploration and the intention to establish an inhabitable human settlement on Mars, it is important to investigate the effects of exposure to space/microgravity and the associated radiations on procreation. Sperm function and integrity are fundamental to male reproduction and can potentially be affected by the environmental changes experienced in space. Therefore, this study was conducted to systematically gather, filter, and collate all the relevant information on the effects of spaceflight on male reproductive parameters and functions. A search was performed utilizing the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. Data were extracted from the major electronic databases including PubMed, and other credible literature sources. MeSH search terms that were employed included "spermatozoa", "microgravity", and "ionizing radiation". The literature search did not discriminate against papers published before a certain date due to the very limited number of articles available. However, there was a restriction on the male gender and language (English). The parameters included in this study are sperm motility, total sperm count, sperm DNA fragmentation hormonal levels and testicular histology. Following a comprehensive literature search, a total of 273 articles were retrieved and screened, 252 articles were excluded due to the irrelevance to the topic, duplication, and non-original articles. A total of 21 articles met the inclusion criteria and are included in the current study. Findings from these studies showed that sperm motility was decreased after exposure to microgravity and ionizing radiation. Total sperm count was also found to be reduced by microgravity only. Sperm DNA fragmentation was increased by both ionizing radiation and microgravity. Testosterone levels and testicular weight were also decreased by microgravity. Although there is a dearth in the literature regarding the effects of microgravity and ionizing radiation on male reproductive parameters, the available findings showed that exposure to microgravity poses a risk to male reproductive health. Therefore, it is essential to develop countermeasures to either manage, treat, or prevent these consequential adverse effects. Hence, this review also highlights some potential countermeasure approaches that may mitigate the harmful effects of microgravity and associated exposures on male reproductive health.

KEYWORDS

spaceflight, microgravity, sperm function, ionizing radiation, male fertility, countermeasures

Introduction

Space is the next habitat of interest that humans are exploring. With the recent agenda of different countries to colonize space, studies investigating the impact of microgravity/spaceflight and its associated exposures on the body system is increasing (Goswami et al., 2012; Goswami et al., 2013; Amjad et al., 2021; Goswami et al., 2021; Harris et al., 2022). Since procreation is an essential part of life sustainability, it is important to investigate the effects of microgravity on male reproduction, and specifically male fertility. One of the earliest studies reported that testes from rats flown on Cosmos 1887 presented with reduced testicular weight when compared to the vivarium controls (Sapp et al., 1990). The findings of Space-Lab three showed a reduction in the number of spermatogonial cells present in the seminiferous tubules of rat testes (Philpott et al., 1985). Following the discoveries of earlier studies, it became pertinent to investigate the exact impact of microgravity and the related exposures on male fertility, if humans are to sojourn in space. Utilizing spaceflight and simulated microgravity, studies have provided more insight regarding the impact thereof.

Hindlimb unloading or the tail suspension model is routinely used in animals to simulate the effects of gravity unloading on different physiological systems (Gao et al., 2018; Hawliczek et al., 2022). Using the tail suspension rat model, Hadley et al. reported that after 7 days of rat suspension without ligation of the inguinal canal, the architectural structure of the testis and epididymis were altered, as the seminiferous tubules became disorganized. Moreso, there was an accumulation of large multinucleated cells, and spermatids were seen in the lumen of epididymis (Hadley et al., 1992). The authors further reported an increase in the levels of serum luteinizing hormone (LH) and follicle-stimulating hormone (FSH), and a concomitant decline in serum testosterone and prolactin levels in the tail suspended rats. The presence of the accumulated immature spermatozoa in the epididymis suggests a possibility of the spermatids having not fully undergone the process of spermiogenesis, where there is an elongation of the nucleus and the condensation of chromatin. The latter is a consequence of the replacement of histones by arginine- and cysteine-rich protamine during spermiogenesis. Additionally, Zhang et al., using a rotating cell culture system, reported that spermatogonial stem cells (SSCs) co-cultured with Sertoli cells exhibited enhanced proliferation surpassing those cultured in static conditions, despite that the SSCs in simulated microgravity underwent an initial lag. After 14 days of culture, proliferating SSCs under simulated microgravity remained undifferentiated, although they maintained clone-forming capacity (Zhang et al., 2014). Meanwhile, another study reported that the number of duplicating cells in the tubules were significantly increased under simulated microgravity after testicular fragments isolated from prepubertal rats were cultured for 3 days in the rotating cell culture system (Ricci et al., 2004). Comparing the two findings, it is clear that simulated microgravity may enhance the proliferation of SSCs within the first few days of exposure but, the question remains if these cells would be able to undergo differentiation. Furthermore, Usik and Ogneva exposed mice to spaceflight for 21–24 days to measure the epigenetic changes. There were no differences observed in the level of cytoskeletal proteins, sperm-specific proteins and biomarkers for epigenetic changes. However, there were changes in the gene expression levels, as there was an increase in demethylase *Tet2* and a decrease in the histone deacetylase *Hdacl* (Usik and Ogneva, 2018). This suggests that spaceflight may influence gene expression since the environment is known to affect genes. Other studies have also reported the impact of space flight/simulated microgravity on sperm functions and sperm parameters (Ogneva et al., 2020a; Ogneva, 2021). In addition to the effects of spaceflight and simulated microgravity on male fertility, studies have reported the impact of some of the exposures that come with microgravity, such as ionizing irradiation, on male fertility (Kamiguchi and Tateno, 2002; Barber et al., 2006; Santiso et al., 2012; Kodaira et al., 2017; Fuller et al., 2019; Wdowiak et al., 2019). Yan et al. reported that sperm DNA fragmentation and the expression of pro-apoptotic biomarkers were significantly higher in the groups exposed to microgravity and irradiation (Yan et al., 2013). Since microgravity and the accompanying exposures such as ionizing radiation may impact male fertility, this study aimed to analyse the literature systematically, bringing into light the evidence of the effect of spaceflight and ionizing radiation on sperm function and ultimately male fertility, putting both animal and human studies into consideration.

Methods

A systematic review was conducted on the effect of space on sperm function and integrity in males using the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (PRISMA, 2020). The literature search did not discriminate against papers published before a certain date. This is due to the very limited number of articles available in the scientific literature on the effect of space flight on male reproductive parameters and sperm parameters. There was a restriction to the male gender, and articles written in the English language. Studies analyzed include human observational studies, animal models and *in vitro* experimental studies. Papers were

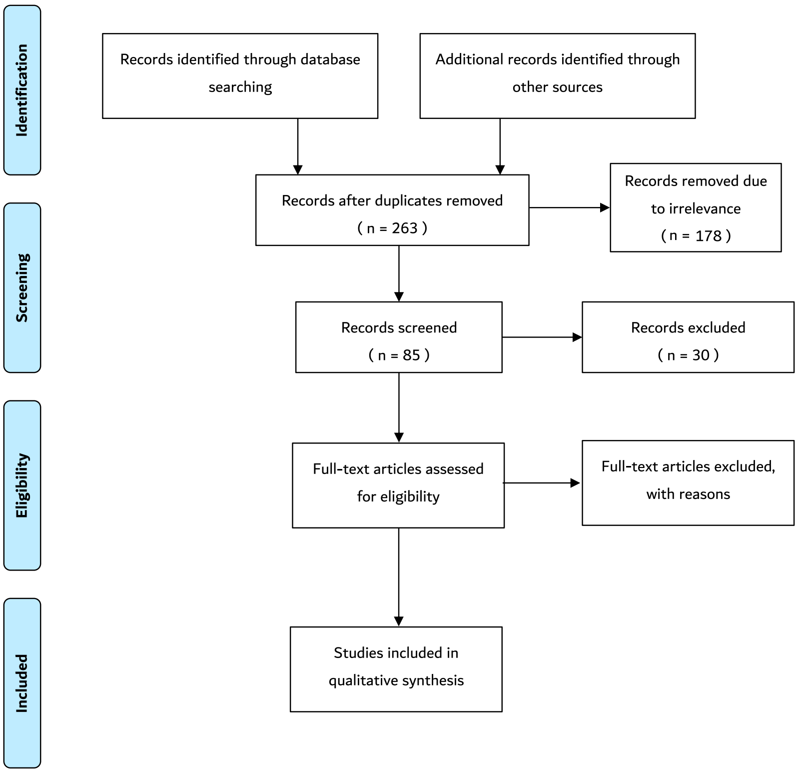

FIGURE 1
The schematic representation of the search method. Following the initial search, 273 records were retrieved. Ten duplicates were removed. Subsequently, 178 records were removed due to irrelevance after the titles were screened. Thereafter, 30 records were excluded due to the irrelevance of the abstracts. Finally, 34 full-text articles were removed because some did not satisfy the inclusion criteria and the others were not original articles. Thus, 21 articles that satisfied the inclusion criteria were included in the study. N = number.

excluded due to duplications, review articles, the lack of proper scientific basis or reliability of data collection.

Data extraction was conducted using the major electronic databases including PubMed, PubMed Central, MedLine, Google Scholar and Cochrane. In addition, other credible literature sources were also explored. Structured literature searches were performed based on specific keywords and inclusion criteria. MeSH search terms and keywords that were used include "sperm", "space", "space flight", "sperm function", "sperm motility", "microgravity", "space radiation", "human body", "spermatozoa", "ionizing radiation", and any synonyms of these words. Boolean operators–AND, OR, NOT–were also used. The title and abstract were screened at first to confirm that the topics match. Thereafter, the entire article was screened to further confirm the relevance of the article to the current study. Data extracted from each paper satisfying the eligibility criteria were used for this study. The variables include study author and publication year, study setting, sample characteristics, studies exploring the effect of cosmic/ionizing radiation on sperm parameters, studies exploring effects of anti/micro-gravity on sperm parameters, and any other study that showed the effects of environmental changes of space on sperm. The PRISMA flowchart summarizing the data collection process is presented in Figure 1.

Results

During the initial search, 273 records were identified. Ten duplicates were removed. Subsequently, 178 records were removed due to irrelevance after the titles were screened. Thereafter, 30 records were excluded due to the irrelevance of the abstracts, such as studies that are not within the scope of the study. Finally, 34 full-text articles were removed because some did not satisfy the inclusion criteria and the others were not original articles. Thus, 21 articles that satisfied the inclusion criteria were added in the current study. A summary of the key information from these articles is shown in Table 1. These articles included five studies from Japan (23.8%), four studies from China (19.04%), four studies from Russia (19.04%), three studies from the United States (14.2%), one study from India

TABLE 1 Overview of findings included in the current study. Studies are grouped into animal and human section. N/A = not available, M = male, LH = luteinizing hormone, FSH = follicle-stimulating hormone, g= gram, kg = kilogram.

Author (year)	Country	Sample size	Subjects	Sex	Age	Average weight	Study setting	*In Vivo/ In Vitro*	Model	Exposure	Results						
											Sperm motility	Total sperm count	Hormones			Sperm DNA fragmentation	Testicular weight and architecture/ histology
													Testosterone	LH	FSH		
Animals																	
Ding et al. (2011)	China	48	Rats	M	8 weeks	200–250 g	Simulation on Earth	*In Vivo*	Tail suspension	Simulated Microgravity	N/A	Decreased	N/A	N/A	N/A	N/A	N/A/Disorganized
Fuller et al. (2019)	United Kingdom	72	Echinogammarus Marinus	M	Unknown	Unknown	Simulation on Earth	*In Vivo*	Beta emitter phosphorus-32	Ionizing Radiation	N/A	No change	N/A	N/A	N/A	Increased	N/A
		72	Gammarus Pulex	M							N/A	No change	N/A	N/A	N/A	N/A	N/A
Kamiya et al. (2003)	Japan	34	Mice	M	≥10 weeks	25.6 g	Simulation on Earth	*In Vivo*	Tail suspension	Simulated Microgravity	Decreased	N/A	Decreased	N/A	N/A	N/A	Decreased/ Disorganized
Li et al. (2013)	China	36	Mice	M	12 weeks	30–35 g	Simulation on Earth	*In Vivo*	Carbon ion beam irradiation	Ionizing Radiation	N/A	N/A	N/A	N/A	N/A	N/A	Disorganized
Masini et al. (2012)	United States	10	Mice	M	8 weeks	Unknown	ISS	*In Vivo*	Space	Microgravity	N/A	N/A	N/A	N/A	N/A	N/A	Disorganized
Matsumura et al. (2019)	Japan	12	Mice	M	5 weeks	Unknown	ISS	*In Vivo*	Space	Microgravity	Decreased	No change	N/A	N/A	N/A	N/A	No change
Olejnik et al. (2018)	Australia	12	Ram Lambs	M	14 weeks	22.6 kg	Simulation on Earth	*In Vivo*	Linear accelerator producing photon beam	Ionizing Radiation	N/A	Decreased	N/A	N/A	N/A	N/A	No change/ Disorganized
					20 weeks	27.5 kg					N/A	Decreased	N/A	N/A	N/A	N/A	No change/ Disorganized
Ogneva et al. (2020)	Russia	Unknown	Mice	M	Unknown	Unknown	Simulation on Earth	*In Vitro*	Random positioning machine	Simulated Microgravity	Decreased	N/A	N/A	N/A	N/A	N/A	N/A
Ogneva et al. (2020)	Russia	560	Fruit Fly	M	2 days	Unknown	Simulation on Earth	*In Vivo*	Random positioning machine	Simulated Microgravity	Increased	N/A	N/A	N/A	N/A	N/A	N/A
Ogneva et al. (2021)	Russia	Unknown	Fruit Fly	M	2 days	Unknown	Simulation on Earth	*In Vitro*	Random positioning machine	Simulated Microgravity	Increased	N/A	N/A	N/A	N/A	N/A	N/A
		21	Mice	M	2 weeks						Decreased	N/A	N/A	N/A	N/A	N/A	N/A
Said et al. (2020)	Egypt	Unknown	Rats	M	Unknown	120–150 g	Simulation on Earth	*In Vivo*	Gamma Cell-40 irradiator (Cesium-137)	Ionizing Radiation	Decreased	N/A	Decreased	N/A	N/A	N/A	Decreased/ Disorganized
Sasaki et al. (2004)	Japan	15	Mice	M	Unknown	25.8 g	Simulation on Earth	*In Vivo*	Tail suspension	Simulated Microgravity	N/A	N/A	Decreased	N/A	N/A	N/A	No change
Tash et al. (1999)	United States	Unknown	Sea Urchin	M	Unknown	Unknown	Space Shuttle Missions	*In Vitro*		Simulated Microgravity	No change	N/A	N/A	N/A	N/A	N/A	N/A
Tash et al. (2002)	United States	Unknown	Rats	M	13–15 months	Unknown	Simulation on Earth	*In Vivo*	Tail suspension	Simulated Microgravity	N/A	Decreased	No change	No change	No change	N/A	Decreased/ Disorganized
Usik et al. (2018)	Russia	42	Mice	M	Unknown	28 g	Simulation on Earth	*In Vivo*	Tail Suspension	Simulated Microgravity	No change	Decreased	N/A	N/A	N/A	N/A	Decreased
Wakayama et al. (2017)	Japan	12	Mice	M	3 months	Unknown	ISS	*In Vitro*	Freeze-dried spermatozoa	Ionizing Radiation	N/A	N/A	N/A	N/A	N/A	No change	N/A

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TABLE 1 (*Continued*) Overview of findings included in the current study. Studies are grouped into animal and human section. N/A = not available, M = male, LH = luteinizing hormone, FSH = follicle-stimulating hormone, g= gram, kg = kilogram.

Author (year)	Country	Sample size	Subjects	Sex	Age	Average weight	Study setting	*In Vivo*/ *In Vitro*	Model	Exposure	Results						
											Sperm motility	Total sperm count	Hormones			Sperm DNA fragmentation	Testicular weight and architecture/ histology
													Testosterone	LH	FSH		
Yan et al. (2013)	China	42	Mice	M	10 weeks	30–35 g	Simulation on Earth	*In Vivo*	Tail suspension and Carbon Ion beam irradiation	Simulated Microgravity and Ionizing Radiation	N/A	Decreased	N/A	N/A	N/A	Increased	N/A
Humans																	
Boada et al. (2020)	Spain	15	Humans	M	26–40 years	Unknown	Simulation on Earth	*In Vitro*	Parabolic flight	Simulated Microgravity	No change	No change	N/A	N/A	N/A	No change	N/A
Ikeuchi et al. (2005)	Japan	18	Humans	M	22–40 years	Unknown	Simulation on Earth	*In Vitro*	Parabolic flight	Simulated Microgravity	Decreased	N/A	N/A	N/A	N/A	N/A	N/A
Kumar et al. (2013)	India	134	Humans	M	21–50 years	Unknown	Earth	*In Vivo*	Occupationally exposed	Ionizing Radiation	Decreased	No change	N/A	N/A	N/A	Increased	N/A
Zhou et al. (2016)	China	118	Humans	M	28.26 ± 3.12 years	78.13 kg	Earth	*In Vivo*	Occupationally exposed	Ionizing Radiation	Decreased	No change	N/A	N/A	N/A	Increased	N/A

Note: N/A = not available, M = males, ISS, international space station.

(4.76%), one study from the United Kingdom (4.76%), one study from Australia (4.76%), one study from Egypt (4.76%), and one study from Spain (4.76%) (Tash and Bracho, 1999; Tash et al., 2002; Kamiya et al., 2003; Sasaki et al., 2004; Ikeuchi et al., 2005; Ding et al., 2011; Kumar et al., 2013; Li et al., 2013; Yan et al., 2013; Zhou et al., 2016; Wakayama et al., 2017; Olejnik et al., 2018; Usik and Ogneva, 2018; Fuller et al., 2019; Matsumura et al., 2019; Ogneva et al., 2020a; Boada et al., 2020; Ogneva et al., 2020b; Said et al., 2020; Ogneva, 2021). Seventeen of the articles conducted the experiment on Earth (fifteen simulations and two occupational exposures), three of the studies performed the experiment on the International Space Station (ISS), and one of the articles conducted experiments during a Space Shuttle mission. Seventeen of the articles included experiments performed on animals, which include mice (N = 224), rats (N = 48), echinogammarus marinus (N = 72), Gammarus Pulex (N = 72), rams (N = 12), and *Drosophila melanogaster* (N = 560). The remainder of the articles included experiments conducted on humans (N = 285). All articles mentioned the age group except for six articles (Sasaki et al., 2004; Xi et al., 2016; Fuller et al., 2019; Ogneva et al., 2020a; Ogneva et al., 2020b; Said et al., 2020). Four articles did not mention the sample size (Tash et al., 2002; Ogneva et al., 2020b; Said et al., 2020; Ogneva, 2021), but in the remaining studies, the reported sample sizes ranged between 12 and 560 subjects. Thirteen articles were exclusively performed on microgravity (Tash and Bracho, 1999; Tash et al., 2002; Kamiya et al., 2003; Sasaki et al., 2004; Ikeuchi et al., 2005; Ding et al., 2011; Masini et al., 2012; Usik and Ogneva, 2018; Matsumura et al., 2019; Ogneva et al., 2020a; Boada et al., 2020; Said et al., 2020; Ogneva, 2021), while seven articles were performed on ionizing radiation (Kumar et al., 2013; Li et al., 2013; Zhou et al., 2016; Wakayama et al., 2017; Olejnik et al., 2018; Fuller et al., 2019; Ogneva et al., 2020b), and only one article conducted combined experiments on both microgravity and ionizing radiation (Yan et al., 2013).

Discussion

Evidence of the effect of spaceflight/ microgravity and ionizing radiation on sperm function: Animal studies

Sperm motility

Kamiya et al. performed a study on 34 mice using the tail suspension method to simulate the microgravity environment of space. The tail suspension method is a test in which the hind limbs of mice/rats are suspended by taping their tails to a raised bar, in such a position that they cannot escape or hold on to nearby surfaces (Can et al., 2011), thus creating a simulated microgravity environment. After 7 days of exposure to microgravity, there was a significant decrease in the average path velocity (31.2 ± 10.7 μm/s versus 42.0 ± 6.5 μm/s), mean track speed (54.7 ± 18.8 μm/s versus 68.1 ± 10 μm/s), and mean progressive velocity (18.3 ± 8.8 μm/s versus 24.5 ± 5.4 μm/s), when compared to the control. These are sperm kinematic parameters, which are used to evaluate the percentage of sperm motility (Kamiya et al., 2003). Matsumara et al. caged 12 mice in the ISS for 35 days, thereafter, spermatozoa were retrieved for *in vitro* fertilization (IVF), to evaluate the effects of microgravity on fertilization. Some parameters of sperm motility were reduced, but that did not affect the ability of the sperm to fertilize the oocyte (Matsumura et al., 2019). In another study, the sperm samples of the experimental mice were isolated from the epididymis and were subjected to simulated microgravity using a random position machine for 1 h, 3 h, and 6 h. The random positioning machine provides 3D multidirectional rotation relative to gravity vector, so that the superposition of the orientation vectors of the objects in the gravitational field is equal to zero per minute, on average (van Loon, 2007). Under the microgravity exposure, the speed of movement did not change after 1 h and 3 h, but it significantly decreased by 32% after 6 h of exposure $p < 0.05$ (Ogneva et al., 2020a). From the study of Ogneva et al., the sperm motility of 2-day old fruit flies and 21 male mice (12 weeks) was evaluated after exposure to a random positioning machine for 6 h. The results obtained indicated that stimulated microgravity leads to a decrease in the speed of movement of mouse spermatozoa by 29% ($p < 0.05$). In contrast, the speed of sperm of the fruit fly after exposure to simulated gravity increased significantly by 30% (Ogneva, 2021). This is also supported by another study by the same author, which was carried out on 560, 2-day old, fruit flies, exposed to a random positioning machine. The results also showed an increase in the speed of movement of the sperm tails by 34% (58.5 ± 2.6 μm/s, $p < 0.05$) (Ogneva et al., 2020b). We can see that the motility of the spermatozoa of insects and mammals under microgravity conditions changes in different ways. It is assumed that the effect of simulated microgravity on the motility of mammalian spermatozoa is mediated through the regulation of phosphorylation and that of insects through the regulation of dephosphorylation of motor proteins that form the axoneme of the sperm tail (Tash and Bracho, 1999; Ogneva, 2021). Nevertheless, Tash et al. who obtained the sperm of male sea urchins and sent them on a Space Shuttle mission for microgravity exposure (Tash and Bracho, 1999), as well as Usik et al. who subjected 42 mice to 30 days of tail suspension to simulate weightlessness (Usik and Ogneva, 2018), reported that there were no significant changes in the sperm motility. The reason for this controversy is not well understood, as there is still a lot of uncertainty and much to be explored.

Regarding irradiation and its effect on sperm motility, Said et al. investigated the underlying mechanism of alpha-lipoic acid (LA) against testicular damage caused by irradiation. The whole body of male rats were exposed to radiation and then administered LA. When rats were exposed to radiation, the sperm motility results showed a significant decrease compared to that of the controls (Said et al., 2020).

Sperm count

Yan et al. used the tail suspension method and carbon ion beam irradiation to simulate a microgravity environment and space ionizing radiation, respectively and investigated its effect on the testis. The carbon ion was equipped with a passive beam delivery system (Li et al., 2007). Compared with the control group, a disordered arrangement of spermatogenic cells was observed and the number of spermatids was significantly smaller in the group exposed to both microgravity and ionizing radiation ($p < 0.05$) (Yan et al., 2013). In the study of Olejnik et al., three irradiation doses (9, 12, 15 Gy) were administered to 24 ram lambs, aged 14 weeks (Group 1, n = 12) and 20 weeks (Group 2, n = 12), by linear accelerator producing photon beam, then testicular biopsies were collected 1,2, and 3 months after irradiation. At 1 month after irradiation, the total sperm count of both groups were significantly decreased $p < 0.05$. Two months post irradiation, the total sperm count of both groups were similar to controls. After 3 months of radiation, Group 1 had less spermatogonia per cross section than controls (~3% versus 10%, $p < 0.05$). Group 2, the higher radiation treatment group (12Gy) had lower spermatogonia per cross section compared to the controls (9.6% versus 12.7%, $p < 0.05$) (Olejnik et al., 2018). The initial large reduction in the numbers of spermatogonia were not permanent as recovery began at 2 months after irradiation. The pattern of change in spermatogonia number over time differed between the two groups. Spermatogonia number in Group 2 decreased rapidly and then increased steadily over the next. 2 months to become similar to the controls, but spermatogonia numbers from Group 1 increased at 2 months after irradiation and then decreased again and was significantly lower than that of controls at 3 months. This pattern of increase and then decrease is also reported by Kangasniemi et al. (Kangasniemi et al., 1996). The reason behind this may be due to the fact that radiation may have induced a defect in spermatogonial stem cells that limits self-renewal to repopulate depleted seminiferous tubules. In the study of Usik et al., 42 mice were subjected to 30 days of tail suspension to simulate weightlessness. Results obtained showed that number of sperm decreased significantly by 45% ($p < 0.05$) (Usik and Ogneva, 2018). This may be due to damage to the testes architecture which will further be discussed in the "testicular architecture/histology" section. In contrast, a study that was performed on the ISS evaluated the total sperm count. Results showed no significant differences, as it was comparable to that of the control group (Matsumura et al., 2019). This finding is consistent with Fuller et al., where two types of male crustaceans, Echinogammarus marines and Gammarus pulex, were exposed to ionizing radiation by beta emitter phosphorus-32 at dose rates of 0, 0.1, 1, and 10 mGy/d. Sperm quality parameters were assessed using a fluorescent staining method.

In E. marines as well as Gammarus pulex, no significant effect of radiation dose rate on sperm numbers was recorded (Fuller et al., 2019).

Sperm DNA fragmentation

The study of Yan et al. used tail suspension and carbon ion beam irradiation to simulate microgravity and ionizing radiation respectively on 42 mice. It was reported that the SDF index was significantly increased in the animals from the experimental group compared to those in the control group (21.35 ± 0.78% versus 9.54 ± 0.31%, $p < 0.001$) (Yan et al., 2013). The SDF index refers to the percentage of sperm with DNA strand breaks in comparison to the total number of sperm analysed (Xi et al., 2016). In addition, Fuller et al., who obtained two types of male crustaceans and exposed them to ionizing radiation by beta emitter phosphorus-32 recorded the occurrence of DNA damage. DNA damage was assessed using single cell gel electrophoresis. Radiation dose rate had a significant $p < 0.05$ effect on DNA damage in E. marines spermatozoa. DNA damage increased as dose rate increased (10 mGy/d treatment 20.69 ± 12.74% versus 10.30 ± 7.51%). No DNA damage assessment was done on Gammarus pulex (Fuller et al., 2019). However, in Wakayama et al., freeze-dried mouse spermatozoa of 12 mice were held on the ISS for 9 months at −95°C and then examined after exposure to space radiation. During the study period, irradiation from space to the sample case was ~100 times higher than that on Earth (Yatagai et al., 2011). The DNA damage in the space sperm samples was slightly increased compared with the control samples kept on Earth, irrespective of mouse strain, but this was regarded as insignificant since the slight damage in the sperm DNA during space preservation was repaired by the oocyte cytoplasm during IVF and did not impair the birth rate or normality of the offspring (Wakayama et al., 2017).

Hormone levels

Kamiya et al. also examined the changes in testosterone levels by using the tail suspension method to evaluate the possibility of spermatogenesis failure in a microgravity environment (Kamiya et al., 2003). The tail suspension model is considered to be a model of body fluid shift (Royland et al., 1994), and a few studies have used tail suspended rats as a spermatogenesis failure model. To date, these studies have demonstrated decreases in the serum testosterone level (Hargens et al., 1984; Hadley et al., 1992). The testosterone level was significantly lower in the tail-suspended group (0.74 ± 1.28 ng/ml, $p < 0.05$) compared with controls (2.38 ± 3.50 ng/ml) in this study. The decrease in testosterone corresponded with the finding of Merrill et al., who examined changes in the serum electrolyte and hormone levels in spaceflight and tail-suspended rats (Merrill et al., 1992). The decrease in testosterone suggests that testosterone secretion is reduced because of a reduction in the testicular blood flow associated with the cranial shift of body fluids (Murashev et al., 1988). In addition to the previous study, Sasaki et al. that used tail suspension methods as well to simulate microgravity on 15 mice noticed that testosterone levels were also decreased (tail suspended group: 0.67 ± 1.21 ng/ml, control group: 3.35 ± 5.09 ng/ml, $p < 0.05$) (Sasaki et al., 2004). Said et al. investigated the underlying mechanism of alpha-lipoic acid (LA) against testicular damage caused by irradiation. Exposure to radiation triggered a significant decrease in both estradiol and testosterone levels by 48% and 64% respectively compared to the control group. Treatment with LA significantly increased estradiol levels; however, it had no effect on testosterone levels (Ogneva et al., 2020b). Nonetheless, in the study from Tash et al., circulating testosterone, FSH, and LH levels were normal at 6 weeks in the tail suspended animals and not significantly different from free-roaming control animals. The results were as follows: testosterone (tail suspended rats at 1,347 ± 156 pg/ml versus control at 1,099 ± 5 80 pg/ml, $p < 0.001$), FSH (tail suspended rats at 119 ± 15 ng/ml versus controls at 162 ± 38 ng/ml), and LH (tail suspended rats at 7.6 ± 1.0 ng/ml versus controls at 6.9 ± 1.6 ng/ml). In this study, tail suspended rats were partly ligated at the inguinal canal to prevent the testes from descending into the abdomen and thus diminishing the effect of the tail suspension (Tash et al., 2002).

Testicular weight and architecture

Ding et al. used the tail-suspension model to simulate microgravity and investigated the effect of microgravity on the tissue structure and function of the testis in sexually mature male rats. Tail suspension was achieved with a tail harness suspending the limbs above the floor of the cage according to the method of Wronski and Morey-Holton (Wronski and Morey-Holton, 1987). Forty-eight male Wistar rats weighing 200–250 g were randomly assigned to three groups (N = 16 each): control, tail traction, and tail suspension. In the tail traction group, the rats were places in a tail-lift harness without suspension of the hindlimbs above the floor. After the rats were suspended for seven or 14 days, the testes were evaluated by histological and electron microscopic methods. Results of the control and the tail suspended rats were included. Upon histopathological observation of the testis by light microscopy, there were almost no observation of spermin the seminiferous tubules. Degeneration and necrosis of the spermatogenic cells were seen, seminiferous tubules were arranged sparsely, surface membrane appeared rough and disordered, interstitial tissue showed edematous, fibrotic, and haemorrhagic changes. Tail suspension caused severe damage to the seminiferous tubules making the basal lamina appear rough. Ding et al. also mentioned that with extended suspension time, the damage to the testes became more serious even reaching irreversibility (Ding et al., 2011). Tash et al. who used the tail suspension method to produce a microgravity environment showed a significant reduction in the number of testicular sperm and elongating spermatids accompanied the decline in testicular weight in the tail suspended animals (1.10 ± 0.11 g, $p < 0.001$) versus the control group (1.70 ± 0.06 g). After 6 weeks of tail suspension, spermatogenesis was significantly reduced to the extent that no spermatogenic cells beyond round spermatids were present in the testis and no normal mature spermatozoa were found in the epididymis (control: 7.82 ± 0.46 sperm x 106/ml, tail suspended: 1.04 ± 0.24 sperm x 106/ml) (Tash et al., 2002). Kamiya et al. simulated the microgravity environment of space and observed that after 7 days of tail suspension, testicular weight was significantly different between the tail suspended mice (93.22 ± 11.31 mg, $p < 0.05$) and control mice (98.53 ± 12.17 mg); however, body weight did not change (Kamiya et al., 2003). This study also looked at the histological architecture of the testes in depth using the light microscopy with hematoxylin and eosin (H&E) and periodic acid–Schiff (PAS) staining. The microscopy showed impairment of spermatocytes beyond the pachytene stage (third stage of prophase meiosis). Almost no spermatozoa were found in the lumen. Multi-nucleated giant cells were occasionally seen. The histology showed hypo-spermatogenesis, as well as loss of all spermatogenic cells, including spermatogonia. The histologic appearance of the Sertoli cells and interstitial Leydig cells appeared to be unaffected in hind limb suspended animals. Another study reported that after Wistar rats were flown for 22 days onboard the biosatellite Cosmos-605, there were no morphological changes in the testes after 24–48 h and 26–27 days postflight, and that the offspring of male rats that were exposed to 22-days weightlessness did not differ from the controls with respect to the number of the new-born, birthweight, weight gain during the first postnatal month, and resistance to hypoxia (Plakhuta Plakutina et al., 1976). However, Macho et al. reported an increase in plasma corticosterone and insulin levels in male rats after space flights for a period of 7, 15, 18 and 20 days. Plasma levels of growth hormone were decreased and those of epinephrine and norepinephrine were elevated in rats exposed to longer space flights (18 or 20 days). This suggests that exposure of rats to space flight is followed by changes in plasma metabolic hormonal levels, but the sympathetic-adrenomedullary system is only slightly activated by longer space flights (Macho et al., 1993). Li et al. investigated the mechanism of action of heavy ion radiation on mouse testes. The testes of 36 male mice aged 12 weeks subjected to whole body irradiation with carbon ion beam (0.5 and 4 Gy) were analyzed at 7 days after irradiation. The histological changes showed cavity formation, disarranged spermatogenic cells, and disrupted basement membrane, disordered and shrunk seminiferous tubules, and thinning seminiferous epithelium (Li et al., 2013). Masini et al. housed 10 mice in the Mouse Drawer System (MDS) developed by the Thales-Alenia Space Italy (Cancedda et al., 2002). The MDS, loaded with the mice, was launched in the Space Shuttle Discovery within the Space Transport System (STS)-128 mission, on 28 August 2009, for exposure to microgravity. It was then housed in the Japanese Experimental Module (Kibou) on the ISS until its return to the Earth by Space Shuttle Atlantis (STS-129 mission) on 27 November 2009. Only three mice returned to the Earth alive after 91 days of space flight. Testes and the epididymis were sampled bilaterally from each mouse killed by inhalation of carbon dioxide at the Life Sciences Support Facility of Kennedy Space Center within 3–4 h after landing and were either processed or frozen immediately. The sections were stained by the H&E staining method and investigated by using an inverted light microscope. The histology showed degenerative changes. This included disorganization and a slight reduction in the thickness of the spermatogenic cells. In addition, sloughing of cells in the lumen, separation of germ cells from the basal laminae and vacuolation of the germinal epithelium were observed. The interstitial tissue displayed inflammatory exudates. More seminiferous tubules were shrunken and distorted (Masini et al., 2012). From the study of Olejnik et al., three irradiation doses (9, 12, 15 Gy) were administered to 24 ram lambs aged 14 weeks (Group 1, n = 12) and 20 weeks (Group 2, n = 12) by linear accelerator producing photon beam, then testicular biopsies were collected at 1,2, and 3 months after irradiation. During the third month, testicular weights (42 ± 6 g) were similar to that of controls (65 ± 13g, $p = 0.137$). Three months post irradiation, in Group 1, there were less spermatogonia per tubule cross section (3 spermatogonia per cross section at 15Gy, 2.9 at 12Gy, 3.2 at 9 Gy compared to 10 at controls, $p < 0.05$). In Group 2, the 12Gy treatment had less spermatogonia per tubule cross section (9.6 versus 12.7, $p < 0.05$) (Olejnik et al., 2018). Said et al. reported testicular damage caused by Gamma Cell-40 irradiator emitting Cesium-137. Testicular weight was reduced in rats exposed to ionizing radiation compared to controls (1.74 ± 0.16 g versus 2.47 ± 0.27g, $p < 0.05$). Testicular histology showed significant morphological alteration in germinal epithelium of the seminiferous tubules associated with atrophy, reduction in the size of the seminiferous tubules, and vacuolation with few spermatozoa seen in the lumen. Moreover, the germinal epithelium has been detached from the basement membrane. Vascular congestion and increased extracellular matrix in the interstitial spaces accompanied with edematous regions, and haemorrhage were prominent in the irradiated group (Ogneva et al., 2020b). These findings were consistent with previous studies (Li et al., 2013; Usik and Ogneva, 2018). Usik et al.. Who subjected 42 mice to 30 days of tail suspension to simulate weightlessness, observed a decrease in testicular weight of the experimental group (177 ± 16mg, $p < 0.05$) compared to the control group (259 ± 20 mg) (Usik and Ogneva, 2018). Nevertheless, the results of another study showed that there was no difference in the weight of the testes following exposure to microgravity (4.01 ± 0.29 g) compared to the control group (3.82 ± 0.25 g) (Matsumura et al., 2019). These findings were consistent with Sasaki et al. (tail suspended group: 94.2 ± 11.2 mg, control group: 98.5 ± 12.2 mg) (Sasaki et al., 2004).

Evidence of the effect of spaceflight/microgravity and ionizing radiation on sperm function: Human studies

Sperm motility

Parabolic flight is a flight that produces an almost gravity-free state for 20–25 s aboard a jet airplane in parabolic flight at a height of 29,000–21,000 feet (Osborne et al., 2014). Ikeuchi et al. collected semen samples from 18 men and exposed the spermatozoa to parabolic flight. Parabolic flight is a flight that produces an almost gravity-free state for 20–25 s aboard a jet airplane in parabolic flight at a height of 29,000–21,000 feet (Ikeuchi et al., 2005). Following exposure, the percentage of total sperm motility (26.85 ± 21.60% versus 45.33 ± 23.98%, p = 0.004), and mean progressive motility (18.53 ± 17.34% versus 32.15 ± 23.35%, $p = 0.007$) were significantly decreased. Studies of Kumar et al. and Zhou et al., included men who were chronically exposed to ionizing radiation due to their occupation, this includes healthcare workers (Kumar et al., 2013; Zhou et al., 2016). Kumar et al. included 134 male volunteers of which 83 were occupationally exposed to ionizing radiation and 51 were non-exposed control subjects. The motility characteristics were markedly lower between the experimental and the control groups ($p < 0.001$) (Kumar et al., 2013). Zhou et al. included 118 subjects of which 46 men were occupationally exposed to ionizing radiation and 72 men were not (the control group). The occupationally exposed men were from various hospitals with diagnostic radiation facilities (mainly computed tomography) (Zhou et al., 2016). All 46 men had operated the equipment for more than 2 years and were considered to have been chronically exposed to low-dose radiation, as reported by Kumar et al. Sperm motility was significantly lower in the exposed men compared to the non-exposed men (20.85 ± 3.41% vs 25.19 ± 3.60%, $p < 0.001$), sperm morphology was abnormal as well (10.04 ± 3.36% vs 16.71 ± 5.67%, $p < 0.001$) (Zhou et al., 2016). Boada et al. collected sperm samples from 15 normozoospermic healthy donor. These samples were preserved in cryostraws and stored in a secure and specific nitrogen vapor cryoshipper. They were then subjected to parabolic flights with an aerobatic single-engine aircraft capable of providing parabolas of up to 8–9 s of microgravity, which is significantly different from other parabolic flight studies. This methodology was previously described by Perez-Poch et al. (Perez-Poch et al., 2016). The sperm motility of frozen samples exposed to microgravity and control samples showed comparable results. There were no statistically significant differences in the percentage of sperm motility (frozen samples at 21.83 ± 11.69 versus controls at 22.54 ± 12.83) (Boada et al., 2020). Other studies that exposed ejaculated sperm to parabolic flights reported a decrease in total sperm motility (control 45.33 ± 23.98 versus microgravity 26.85 ± 21.60% (p = 0.004), and progressive motility (control 32.15 ± 23.35 versus microgravity 18.53 ± 17.34% ($p = 0.007$)), and thought that the reason behind this might be due to chemical changes in the intracellular environment during microgravity exposure (Ikeuchi et al., 2005). However, Boada et al. suggested that the undetectable differences observed in the parameters analyzed could be that the effects of microgravity on sperm motility was minimized because the samples were frozen and sperm integrity was shielded by cryoprotectants.

Sperm count

Boada et al. reported that the exposure of healthy donors to parabolic flight did not affect sperm count, as no significant changes between the experimental and control groups (39.01 ± 32.02 × 10^6/ml versus 39.29 ± 36.53 × 10^6/ml) was observed. This result reinforces the previous hypothesis that sperm is protected against microgravity by freezing them in comparison to the fresh ones (Boada et al., 2020). Additionally, Kumar et al. showed that after exposure to occupational ionizing radiation, the total sperm count was not affected, as there was no statistically significant difference between the exposed and non-exposed men (64.16 ± 4.40 × 10^6/ml versus 68.44 ± 5.98 × 10^6/ml) (Kumar et al., 2013). This was supported by Zhou et al. who stated that there were no statistically significant differences in sperm concentration between the exposed and the non-exposed men (Zhou et al., 2016).

Sperm DNA fragmentation

Both Kumar et al. and Zhou et al. studied men who were chronically exposed to ionizing radiation due to their occupation and both studies had the same outcome. Kumar et al. reported that the level of SDF was significantly higher in the exposed group as compared to the non-exposed group ($p <$ 0.05–0.0001). These findings showed that exposure to occupational radiation may have a profound implication on the fertility and reproductive outcome of health workers (Kumar et al., 2013; Zhou et al., 2016). Zhou et al. reported that the SDF index of exposed subjects was 29.43 ± 4.57% and non-exposed subjects 14.68 ± 6.32% ($p < 0.001$). These findings show that exposure to occupational radiation may have a profound implication on the fertility and reproductive outcome of health workers, and importantly, on the health of the children born to such fathers since the spermatozoa carrying nuclear abnormalities can fertilize the oocytes (Marchetti et al., 1999), and the embryos thus derived from the irradiated sperm carry substantial risk of trans-generational genomic instability (Shimura et al., 2002; Adiga et al., 2010). In space, there is exposure to ionizing radiation and therefore the exposure could also potentially carry risk of trans-generational genomic instability. On the contrary, Boada et al. showed that there were no significant changes. Samples exposed to microgravity had a mean of 13.33 ± 5.12% sperm fragmentation, while control samples had a mean of 13.88 ± 6.14%. The mean percentage of SDF was similar in both groups (Boada et al., 2020).

Summary

Having critically and systemically analyzed the literature, and deducting evidence from both animal and human studies, it is safe to say exposure to microgravity and associated ionizing irradiation causes hypogonadism, hyposteriodogenesis, decreased sperm function and sometimes transgenerational gene mutation in animals. However, in humans, although studies have shown hyposteriodogenesis as a consequence of exposure to microgravity, report about its effect on sperm quality and quantity are contradictory. Some studies reported decrease in sperm quality such as motility while some showed no changes. This trend is also seen in the reports of sperm quantity such as sperm count and semen volume. These contradicting outcomes on sperm functional parameters may be because of different mode of exposure, method of semen collection (*in vivo* (samples collected from men placed under microgravity) versus *in vitro* (samples collected from healthy donors)), the duration, and the age differences in the different set of cohorts. Similarly, aspects related to social interactions and psychology, which play important roles in reproduction physiology, could also have contributed to the contradictory results. Future studies related to male reproduction should carefully integrate these confounding variables.

Despite these conflicting outcomes on sperm parameters in humans, sex hormones are often reduced, and this can hamper spermatogenesis. To give a perspective on how spermatogenesis can be adversely impacted; under microgravity, the body orientation is not usually straight and the blood flow to the testes maybe further reduced, causing decreased oxygenation. Under a normal erect body position, blood flow to the testis is lesser when compared to other organs (Rawy et al., 2021). The testes receives its blood supply through the testicular artery, having high flow resistance and hence resulting in a lower intra-testicular capillary pressure and slightly higher venous pressure than other organs (Sweeney et al., 1991; Rawy et al., 2021).

Most evidence suggests that the testis is particularly susceptible to vascular system disruption and that testicular dysfunction can result from moderate blood supply disruption (Damber and Bergh, 1992; Bergh and Damber, 1993). As in every other organ, blood supply must be strictly mediated, this is particularly significant for the testis since the oxygen concentration in the seminiferous tubules is very low (Setchell, 1990). Therefore, any decrease in blood supply causes ischemic harm that may lead to sperm deterioration. Partial restriction of the testicular artery has been reported to have an adverse effect on the development, volume and histological structure of bull testes, resulting in complete or incomplete arrest of spermatogenesis (Kay et al., 1992). Several studies in humans (Battaglia et al., 2001; Biagiotti et al., 2002; Tarhan et al., 2003) and rats (Bergh et al., 2001) have shown an association between testicular blood flow and quality of sperm. Moreover, hormones appear to be involved in the mediation of testicular blood flow (Macho et al., 1993; Ogneva et al., 2016; Rawy et al., 2021; Abdelnaby, 2022). This is evidenced by the report of Rawy et al., that following administration of GnRH to rams, the arterial pulsatile index (PI) and resistance index (RI) decreased for about 120 h, and serum testosterone concentration was negatively correlated with both PI and RI (Rawy et al., 2021). This observation was also reported in bulls, with the inclusion that nitric oxide was also increased (Abdelnaby, 2022). Hence, reduced hormonal levels seen upon exposure to microgravity and associated irradiation could cause testicular dysfunction by altering testicular blood flow and disrupting the normal functioning of the HPG axis.

Evidence that exposure to microgravity poses a risk to male reproductive health has been provided thus far. Since it is essential to maintain and preserve fertility for successful space exploration and colonization, countermeasures must be set in place to either manage, treat or prevent any adverse effects that can be imposed due to exposure to microgravity or associated irradiation.

Potential counter measures to the effect of microgravity on sperm function

Researchers have been able to identify some of the adverse effects of microgravity on various physiological systems, and this includes sperm function. In counteracting these negative consequences, studies have focused more on the use of pharmacological methods, exercise, improving dietary supplementation and creating artificial gravity. Though these measures are directed towards improving physiological functions, the question abates whether these strategies can also improve male reproductive health?

Therefore, this section will briefly discuss some of the impending approaches that may be employed to prevent, treat, or alleviate the negative consequences of microgravity on male reproductive health. This includes cryopreservation of sperm before take-off, gene silencing, telomere length preservation, exercise, improved dietary supplementation, inducing artificial gravity, and the provision of devices that may protect against ionizing irradiation.

Cryopreservation

Sperm cryopreservation is extensively used in infertility treatment and fertility preservation in cancer patients, which has consequently resulted in millions of live births from these patients (Yu et al., 2021). Sperm cryopreservation can be performed by either slow freezing or vitrification. Slow freezing has been successfully employed and widely used at fertility clinics performing the different assisted reproductive techniques (ART) (Jang et al., 2017). Vitrification which is a process of solidifying liquid into an amorphous or glassy state has become a faster alternative method of sperm cryopreservation with significant benefits regarding simple equipment and applicability to fertility centers (Tao et al., 2020).

Boada et al. showed in their study that frozen sperm samples preserved in cryostraws and stored in a nitrogen vapour cryoshipper do not suffer significant alterations after microgravity exposure. The results of motility, morphologically normal spermatozoa and the SDF index were comparable between the control and microgravity groups. The comparability may be due to the placement of the cells. The frozen cells were in a near-dormant state, and this may cause reduced cellular metabolism due to the reduced temperature. Therefore, the lack of differences seen between frozen samples exposed to microgravity and those maintained in-ground state affords the possibility of considering the safe transport of human male gametes to space (Boada et al., 2020). Since both gametes and embryos can be cryopreserved, then, in the advent of sojourning in the space, this method can be utilized.

Gene silencing

The application of gene silencing has received great attention in recent years. This phenomenon, 'gene silencing', is utilized as a defense mechanism against invasive nucleic acids. For the knockdown of gene expression, the process of RNA interference (RNAi) must be initiated. RNAi is a biological process by which double-stranded RNA (dsRNA) induces sequence-specific gene silencing by targeting mRNA for degradation (Han, 2018). When RNA are double-stranded, the body regards them as foreign, and are cut into smaller segments (small interfering RNA (siRNA)) by the nuclease Dicer, which then triggers RNAi response to induce gene silencing or degradation. In research, gene-specific, synthetic siRNA can be used to induce gene silencing. In 2001, Elbashir et al. showed that transfection of synthetic 21 base-pair siRNA duplexes into mammalian cells efficiently silences endogenous gene expression in a sequence-specific manner (Elbashir et al., 2001). This finding heralded the use of siRNA for gene silencing in mammalian systems.

This technology can be integrated into the study of microgravity. This can be carried out by initiating RNAi response in genes that their expression under microgravity/ spaceflight or any associated irradiation could cause detrimental effects on male reproductive health. For instance, in the studies of Usik et al. and Ogneva et al. where some genes were mal-expressed upon exposure to microgravity (Usik and Ogneva, 2018; Ogneva et al., 2020a), the process of gene silencing may prove to be a useful tool to alleviate the adverse effects thereof.

Telomere preservation

Telomeres, situated at the end of a chromosome are made of repetitive sequences of non-coding DNA that protect the chromosome from damage. Luxton et al. reported that telomere lengths were increased during spaceflight irrespective of mission duration and that its length rapidly shortened upon return to Earth (Luxton et al., 2020a). Upon completion of the experiment, the telomeres' length of astronauts that were exposed to microgravity was shorter than it previously was. They also showed that telomere length was positively correlated to oxidative stress (OS) and increase frequencies of chromosomal inversions. The same authors reported, in a different study, that in addition to increased OS and inflammation, there was persistent DNA damage observed with telomeric and chromosomal aberrations after exposure to microgravity (Luxton et al., 2020b).

Although studies have not yet identified any association between telomere length and altered male reproduction function. However, the occurrence of OS and inflammation reported under microgravity and accompanying radiation, which are also known mediators in the pathogenesis of male infertility, makes the strategy of preserving telomere length viable. The indirect preservation can be performed by preventing OS through implementing antioxidant therapy in astronauts during spaceflight, as this may counteract the negative effect of OS on telomere length.

Exercise and improved diet

It is widely known that exposure to microgravity induces changes in the physiological functions of the body. In counteracting these adverse effects, it was suggested that exercise and diet supplement implementation may be critical roles (Hides et al., 2019). For instance, Hides et al. suggested that since exercise improves the symptoms of patients with lumbopelvic and spinal muscle pain (low back pain), then exercise should be encouraged in astronauts, since exposure to microgravity causes low back pain and some other skeletal defects.

Furthermore, since sufficient energy intake is required to maintain anabolic processes necessary for normal bone growth support and remodelling, it is suggested that diets that can improve calcium bioavailability be supplemented. Calcium bioavailability can be enhanced by reducing dietary alkalinization, restricting dietary sodium and preventing dietary calcium oxalate urolithiasis. Fettman described in detail the importance of incorporating dietary management when getting exposed to microgravity (Fettman, 2001). These processes of improved lifestyle have been shown to have positive impact on male reproductive function.

Conclusion and recommendation

The current study systematically reviewed the effects of microgravity and ionizing radiation on sperm motility, total sperm count, sperm DNA fragmentation, hormone levels, and testicular architecture/histology, and how it affects male fertility. Although some studies reported the negative effect of microgravity on sperm motility, some showed no effect, while others indicated that normal sperm motility was restored post-exposure. It remains evident that exposure to microgravity and ionizing radiation can adversely affect spermatogenesis and as well alter sperm DNA/chromatin integrity. Since the mature human spermatozoa do not have the capability to repair their DNA, sperm DNA damage depending on the radiation dose may lead to permanent infertility, and/or increase the risk of congenital anomalies occurrence in the offspring.

Recommendations for future research would be to perform experiments and observe the effects of space flight on the male reproductive system during longer space missions utilizing both *in vitro* and *in vivo* studies. Researchers should also begin to perform more experiments on human subjects as part of their studies since these findings will provide a true reflection of the results. With plans for significantly longer space missions and ultimately colonization, it is vital to consider multi-generational survival under microgravity. Furthermore, studies that will focus on developing and implementing countermeasure strategies should be designed, as this will help in reducing the futuristic consequences of exposure to microgravity and associated irradiation.

Author contributions

KA—literature search, drafted manuscript, edited TO—drafted manuscript, reviewed and edited NG—reviewed manuscript HA-reviewed manuscript SSDP—conceptualized and reviewed.

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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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The impact of stance during heel raises on the hybrid ultimate lifting kit (HULK) device: A future microgravity exercise machine

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Extended missions in microgravity, such as those on the International Space Station (ISS) or future missions to Mars, can result in the physiological deconditioning of astronauts. Current mitigation strategies include a regimented diet in addition to resistance training paired with aerobic exercise. With the increased effort toward long duration space missions, there is room to optimize the cost, required time of use, and mass of exercise equipment. This research effort focuses on understanding the biomechanics of Heel Raise (HR) exercises while using the Hybrid Ultimate Lifting Kit (HULK) device, an exercise device designed to optimize volume and functionality. Using the biomechanics tool OpenSim, the effect of HR foot stance (15° inward, 15° outward, and straight) was assessed by analyzing kinematic and kinetic data. In particular, we analyzed peak joint angles, range of motion, joint moments, and angular impulses of a single subject. Preliminary results indicated no significant differences in terms of ankle/metatarsophalangeal/subtalar joint angles, range of motion, joint moments, and angular impulses between foot stances. In addition, loaded HR exercises were compared to body weight HR exercises without the HULK device. Finally, recommendations are made towards an optimal HR routine for long-duration space missions. The impact to health and rehabilitation on Earth is also discussed.

KEYWORDS

biomechanics, opensim®, kinematics, dynamics, microgravity

Introduction

Future spaceflight missions to the Moon and Mars will require extended travel times to and from the mission locations (Mars Architecture Steering Group, 2009). As a result, astronauts will be exposed to long periods of microgravity that can lead to physiological complications impacting the success of these missions. Specifically, the crew will experience the deconditioning of the neurovestibular, cardiovascular, and musculoskeletal systems. For the purpose of this research effort, a focus will be placed on mitigating the well-documented negative effect of prolonged microgravity on the musculoskeletal system (West, 2000; Trappe et al., 2009; Grimm et al., 2016). For example, microgravity has been known to decrease the volume of some muscles by up to 10.3% in as little as 8 days (LeBlanc et al., 1995), or up to 15.4% in 16 days (Akima et al., 2000). The muscles of the calf, (i.e., the gastrocnemius and soleus) have reduced in volume by an average of 17% after longer duration missions, and once back on Earth, these losses can take up to 60 days to recover (LeBlanc et al., 2000). In addition, bone loss can occur at a rate 10 times greater than that of post-menopausal women (Cavanagh, Licata and Rice, 2007), and it is estimated to be up to 1% per month in some cases (Nicogossian et al., 2016). These physiological decrements are magnified when considering that astronauts are expected to perform EVAs upon landing on Mars.

One of the main mitigation strategies to limit the loss of bone and muscle mass is the implementation of exercise protocols (Smith et al., 2012). Due to the weightless environment, creative methods to provide resistive exercise to the crewmembers are imperative. The Hybrid Ultimate Lifting Kit (HULK) device developed by ZIN Technologies is a spaceflight exercise device prototype that utilizes gas cylinders and electric motors to provide resistance to the user (Thompson et al., 2015). The ability to perform squats and deadlifts on the HULK device has previously been studied (Thompson et al., 2019), but the HULK's impact on heel raises is yet unknown. Heel raises will be part of any spaceflight exercise routine and the ability for users to confidently perform them on the HULK device will be critical. Variations in foot stance during heel raise exercises results in changes in muscle activation of the muscles in the lower leg (Riemann et al., 2011; Cibulka et al., 2017), which could be integrated into a training program. In this research effort, we specifically focus on investigating lower body kinematics and dynamics as it relates to foot stance when performing heel raise exercises on the HULK device.

Methods

Testing setup and heel raise exercise configurations

The data for the study were collected from a 68 kg male subject at NASA Glenn Research Center. All testing was done with approval from the Institutional Review Board at NASA Glenn and informed consent from the subject. The subject performed heel raises with the HULK device in four configurations. The first three configurations consisted of heel raises in different foot stances with resistance provided by a t-bar that the subject held with his two hands in front of him. In all three configurations, the resistance transmitted by the t-bar was 75 kg (165lbs). The three different foot stances were as follows: 15° rotated outward, 15° rotated inward, and 0° (i.e., feet straight). In addition to these three configurations, an additional control configuration of heel raises was performed with just body weight as resistance (i.e., no t-bar), and with a straight stance (i.e., feet at 0°) which was designated straight stance without HULK (WH). In this condition, the subject balanced without touching the HULK device (i.e., without external support or load). Each configuration was performed for multiple repetitions, which were averaged for analysis. The number of good quality repetitions in each configuration varied between 3 and 5. Specifically, 5 repetitions were successfully captured during

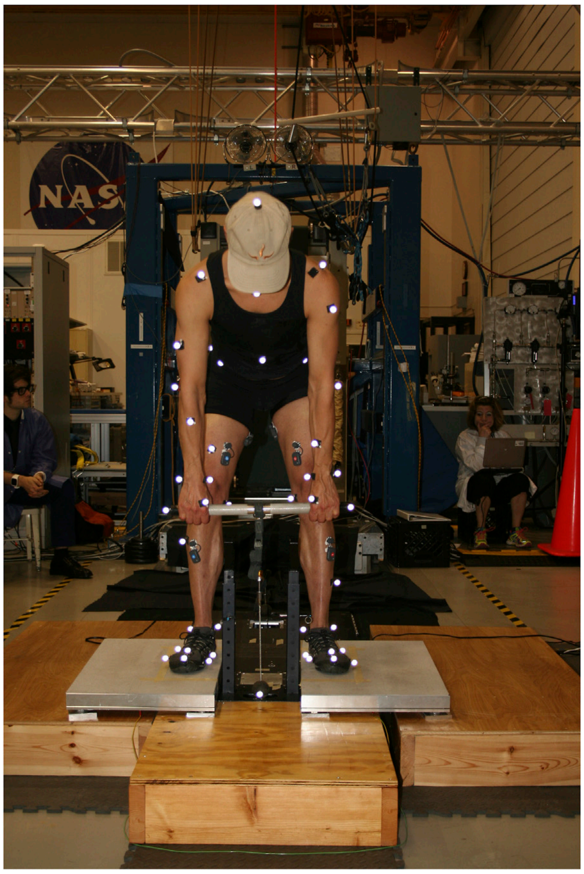

FIGURE 1
The HULK testing setup. The subject is holding the t-bar of the HULK device. In addition, the motion capture markers are seen covering the upper and lower body.

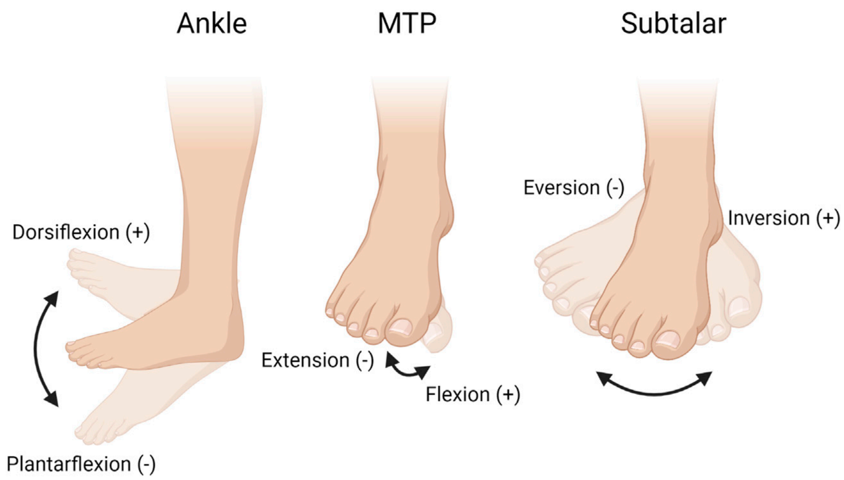

FIGURE 2
The three joints analyzed during the heel raises exercise: the ankle joint (dorsiflexion/plantarflexion), the metatarsophalangeal (MTP) joint (flexion/extension), and the subtalar joint (inversion/eversion). Figure created with BioRender.com.

the 15-degree outward and 0-degree configurations, 4 repetitions were successfully captured during the 15-degree inward configuration, and 3 repetitions were successfully captured during the straight stance without the HULK device. A good quality repetition was determined based on the time to complete the repetition (which was approximately 1 s), full kinematic range of motion, and the completeness of the data from which the repetition was taken.

Motion data of the heel raises were recorded in all configurations using a Smart DX System (BTS Bioengineering, Quincy, MA) at 100 Hz. The motion capture system tracked the movement of 59 reflective markers placed at specific locations on the subject's body and t-bar while performing the heel raises. In addition, force plate data were obtained to record ground reaction forces at 200 Hz which was synchronized with motion capture data. The HULK contained load cells in line with the t-bar cable for measuring the resistance during the exercises. The load cell measurements were also synced with the motion capture and force data. An image of a subject with motion capture markers using the HULK device can be seen in Figure 1.

For the present analysis, we focused on the three joints located within the foot: the ankle joint (dorsiflexion/plantarflexion), the metatarsophalangeal (MTP) joint (flexion/extension), and the subtalar joint (inversion/eversion). These movements are shown in Figure 2. Specific dependent variables include joint angles (including range of motion and peak angle), and joint moments (including angular impulse and peak moment) during each HR configuration. Only the right foot was used for the analysis of angles and moments. Finally, the center of pressure throughout the HR repetitions was also calculated.

OpenSim simulations

To process the motion capture data, we used a biomechanics software called OpenSim. OpenSim has a large variety of capabilities such as inverse kinematics (IK) and inverse dynamics (ID). In addition, the software has proven capable of assessing a variety of motions such as walking (Anderson and Pandy, 2001), running (Hamner, Seth and Delp, 2010), jumping (Anderson and Pandy, 1999), and squatting. The software has also been used to model the effect of spacesuit joint torques (Gilkey, 2012; Diaz and Newman, 2014) and their impact to extravehicular activity (Kluis et al., 2021a; Kluis et al., 2021b). The human model used for our simulations was a full body model developed by Apoorva Rajagopal and modified by researchers at NASA Glenn Research Center (Hicks et al., 2015; Rajagopal et al., 2016). In addition to the human body, a geometry file was loaded into the model to represent the HULK t-bar, which is where the resulting force from the HULK device was applied.

The first step to the analysis consisted of scaling the OpenSim model to match the size of our subject. Using spatial marker data from the motion capture data, we adjusted the anthropometric measurements of our model to match those of the subject. In addition, the mass of the model was adjusted to 68 Kg to match the mass of our subject. With appropriate anthropometric measurements, additional steps were performed to ensure a proper connection of the HULK t-bar (which was also modeled in OpenSim) to the hands of the model, allowing the transfer of forces from the HULK resistive cable to the human model. To accomplish this, two additional markers were added to the t-bar, and these were leveraged to create a point and ball joint between the t-bar and the left and right hands of the scaled OpenSim model, respectively.

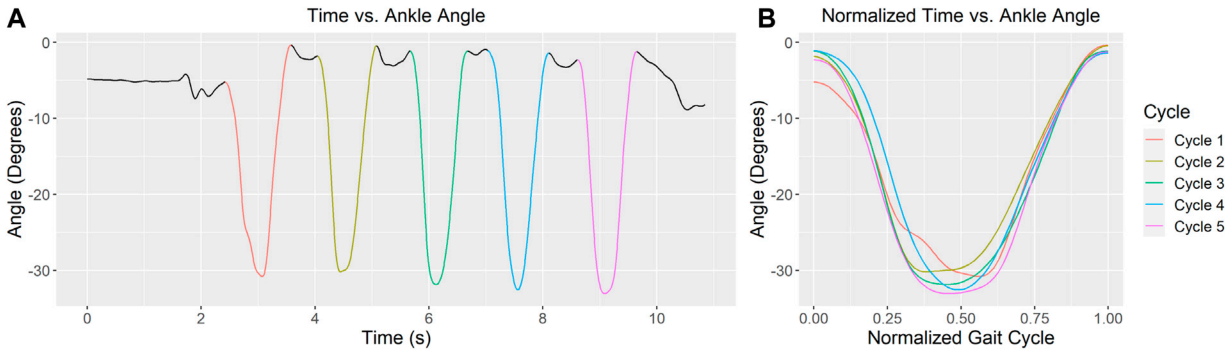

FIGURE 3
(A) Ankle angle during straight foot stance heel raises over time (5 repetitions). Each repetition is marked with a different color. **(B)** Normalized repetitions are superimposed for further analysis.

In the next step, we performed inverse kinematics to calculate joint angles based on the collected motion capture data. The noise associated with our experimental data acquisition was first reduced using a Butterworth filter (cutoff frequency 6 Hz). OpenSim calculates the inverse kinematics by minimizing the (weighted) squared errors between the experimental motion capture markers and a series of virtual markers previously placed on the model. Given the purpose of the study and our specific interests, the markers located on the lower body of the model were given a higher weighting scheme (×50). In addition, the markers located around the HULK TBAR were also weighted higher (×25) to ensure a proper connection between the TBAR and the hands of the model. In the last step, based on the previously calculated kinematics solutions and incorporating the foot and TBAR external forces, we performed inverse dynamics to calculate the net forces and moments at every joint in the model produced during the heel raise exercise.

Data processing and statistical analysis

The joint angles and moments for the right ankle, MTP, and subtalar joints were visually inspected to ensure appropriate quality for further analysis. For each configuration, valid HR repetitions were normalized (to 101 samples, from 0 (the start of the repetition) to 1 (the end of the repetition), averaged, and used for analysis. This process is visually represented in Figure 3.

To compare the stance positions and effects of the HULK device to straight stance repetitions, peak joint angles, range of motion, peak moments, and angular impulses were assessed statistically. First, the data were tested for normality (Shapiro-Wilk test) and heteroscedasticity (Breusch-Pagan test). Some of the data did not comply with these assumptions, and therefore we implemented non-parametric techniques. In particular, a non-parametric Mann-Whitney *U* test was used to compare the inward, outward, and straight foot stances. Similarly, the Mann-Whitney *U* test was also used to compare the straight foot stance with and without the HULK device. A Mann-Whitney *U* test was chosen over a statistical test for dependent variables because we considered that the individual repetitions of a given stance (e.g., first repetition of the straight stance) are not necessarily dependent on the individual repetitions of any other stance (e.g., first repetition of the outward stance). As a result, and acknowledging the limitations related to the small sample size, we chose to use an independent variable statistical test. Significance was set to $\alpha = 0.05$. When comparing the three stance conditions (with HULK), a Bonferroni correction for multiple comparisons was implemented and, in this case, $\alpha = 0.05/3$.

In addition to the analysis of the biomechanics global metrics above, we also investigated differences in kinematics and dynamics during the time course of the HR repetition between the three different foot stances (inward, outward, straight). To compare stances, we performed pairwise comparisons at each normalized time point using a non-parametric Mann-Whitney *U* test. If two stances presented statistically significant differences of at least 10 consecutive time normalized points ($p < 0.05$), then that phase of the heel raise was considered to be significantly different between the foot stances. This method was used in place of an overly conservative Bonferroni adjustment because of the elevated number of pairwise comparisons conducted (101 pairwise comparison, which would yield an $\alpha = 0.05/101$). In addition, this method of analysis was already used in previous HULK studies (Thompson et al., 2019).

Finally, we analyzed the center of pressure of the subject's contact with the force plates. Center of pressure assessments are used to confirm correct placement of the feet through the entire

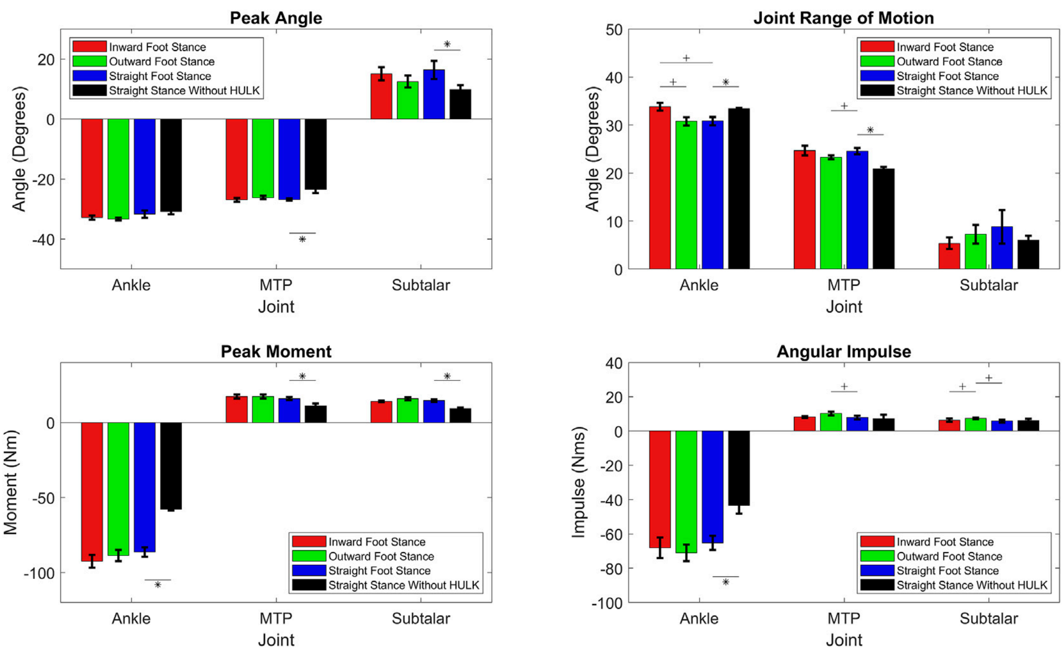

FIGURE 4
Peak angle, range of motion, angular impulse, and peak moment in each one of the three joints investigated (ankle, MTP, subtalar) for inward foot stance, outward foot stance, straight foot stance, and straight stance without HULK conditions. A "+" symbol indicates significant differences between an inward, outward, or straight foot stance (α = 0.05/3), and a "*" symbol indicates significant differences between straight foot stance and straight stance without HULK (α = 0.05). Data presented as average ±SD.

exercise period and identify abnormalities in subject movements. The center of pressures for the left and right feet through the entire exercise period of a given condition were plotted and assessed qualitatively with visual inspection.

Results

Overall metrics: Peak angle, range of motion, peak moment, and angular impulse

Figure 4 summarizes the peak angle, range of motion, peak moment, and angular impulse results for the three joints investigated (ankle, MTP, and subtalar) in each one of the 4 conditions (inward, outward, straight, and straight without HULK). Quantitative values are also summarized in Table A1. Overall, these metrics indicate only minimal changes to kinematics and dynamics because of foot stance with HULK loading. Only a small portion of the joints displayed significant statistical differences. Specifically, most of the statistical difference can be found in the range of motion and angular impulse. For example, there is a significant difference between the ankle range of motions of inward and outward foot stances (p = 0.0159) and inward and straight foot stances (p = 0.0159). The outward and straight foot stance's range of motion are significantly different in the MTP joint (p = 0.0159). Similarly, the outward and straight foot stances (p = 0.0159) and the inward and outward foot stances (p = 0.0159) have significantly different angular impulses in the Subtalar joint. Finally, the angular impulse of the outward and straight stance in the MTP joint are significantly different (p = 0.0159).

Analysis of the straight foot stance with and without the HULK device resulted in numerous metrics that are significantly different. The ankle joint has significantly different range of motion (p = 0.036), peak moment (p = 0.036), and angular impulse (p = 0.036). The MTP joint has significantly different peak angle (p = 0.036), range of motion (p = 0.036), and peak moment (p = 0.036). Finally, the subtalar joint has significantly different peak angle (p = 0.036) and peak moment (p = 0.036).

Biomechanical differences in normalized heel raise cycle between outward, inward, and straight foot stances using the HULK device

The top of Figure 5 shows the average (±SD) joint angles for the ankle (dorsi/plantarflexion), MTP (flexion/extension), and subtalar (inversion/eversion) joints during HR exercises in the three stances considered (outward, inwards, and straight). For the ankle in

Figure 5A, most of the statistically different phases are located at the end of the repetition for all three comparisons. In addition, there is a small region approximately at the 25% of the HR cycle where we found differences between the inward foot stance and straight foot stance. This region represents the ascending phase of the heel raise. Similarly, the MTP angle in Figure 5B shows significant differences between the inward stance and the outward and straight stances at approximately 25% of the HR cycle. Finally, the majority of the subtalar joint angle in Figure 5C shows differences between stances only in the first 25% of the HR cycle, specifically between the outward foot stance and the inward and straight foot stances. In addition, there is also a short phase close to the peak of the HR where we also found significant differences between the outward and inward foot stances.

The bottom of Figure 5 shows the average (±SD) moments for the ankle (Figure 5D), MTP (Figure 5E), and subtalar joints (Figure 5F) during HR exercises in the three foot stances considered (outward, inwards, and straight). In general, none of the joints showed any significant differences between foot stances in the required moments for the HR exercise. One exception is the subtalar moment, were we found differences between the outward and inward foot stance between the 80%–100% of the HR cycle.

Biomechanical differences in normalized heel raise cycle with straight foot stance with and without the HULK device

The top Figure 6 shows the average (±SD) ankle (Figure 6A), MTP (Figure 6B), and subtalar joint angles (Figure 6C) during the HR repetitions for the straight foot stance with the HULK device and for the straight stance without the HULK device. The beginning and end phases of the ankle joint during a HR cycle showed significant differences. In comparison, the MTP angle was only significantly different between the two configurations at approximately the 50% mark of the HR cycle. Finally, the subtalar joint angle showed significant differences between the straight foot stance with and without the HULK device through the raising phase and the end phase of the heel raise cycle.

The bottom of Figure 6 shows the average (±SD) moments for the ankle (Figure 6D), MTP (Figure 6E), and subtalar joints (Figure 6F) during the HR repetitions for the straight foot stance with and without the HULK device. As expected, given the difference in resistance, the majority of the ankle and subtalar moments were significantly different between the two conditions. Specifically, the ankle moment showed significant differences almost throughout the entire HR cycle, except for a small number of time-normalized points at the 25% mark and at the very end of the cycle. Similarly, the subtalar moment exhibited significant differences between both configurations throughout the entire heel raise cycle, except for a few points at the 25% and 75%, marks, as well as at the very end of the cycle. Finally, and in contrast to the other two joints, the MTP moment only presented significant differences between the two conditions during a short period of the HR cycle around the 13% mark.

Center of pressure

Figure 7 shows the progression of the center of pressure through every heel raise cycle for the left and right foot in all three stances with the HULK device and straight stance without the HULK device. Four markers were located on each foot, and their location is also shown in the figures. Finally, center points for the inside/outside markers and toe/heel markers are shown next to the centroids of the center of pressure. In general, the center of pressure maps look as expected, with the centroid for each stance being located toward the "ball" of the foot. The figures are also useful for visually comparing the magnitude of difference in stance positions.

Discussion

The overall metrics for the three stances with the HULK device have differences in only the range of motion and angular impulse. Specifically for the range of motion, the inward foot stance is significantly different than the outward and straight stances in the ankle joint, and the outward foot stance is significantly different than the straight stance in the MTP joint. For angular impulse, the outward foot stance is significantly different than the inward and straight stances in the MTP joint, and the outward stance is significantly different than the straight stance in the subtalar angle. The small number of significantly different metrics over all three stances with the HULK device are indicative of the minimal impact that stance has on the peak angle, range of motion, peak moment, and angular impulse of the joints in the lower leg during HR exercises. In comparison, the straight stance with and without the HULK device indicated a significant impact from the HULK device to the peak angle, range of motion, peak moment, and angular impulse metrics of the subject. This was an expected result as additional resistance to the exercise will add forces and moment throughout the lower body, and as a result, impact the overall kinematics of the exercise.

Generally, we found minor changes in kinematics and dynamics between stances. The majority of the statistically significant differences in the kinematic cycles can be identified at the beginning and end of the HR exercise cycle. This is likely due to the choice of repetition start and end time and not a result of the stance change. For example, lengthening the entire repetition time by choosing an earlier overall start time can result in an initial angle that affects the mean and standard deviation for the given time normalized point. This issue is unavoidable as any choice of start or end point will have impacts to the overall kinematic curve of the stance and joint combination. As previously specified, preference was given to matching peak kinematic angles as these were assumed to be more sensitive to the impact of stance change. While the joint kinematics have several areas of statistical difference, the joint moment had notably few

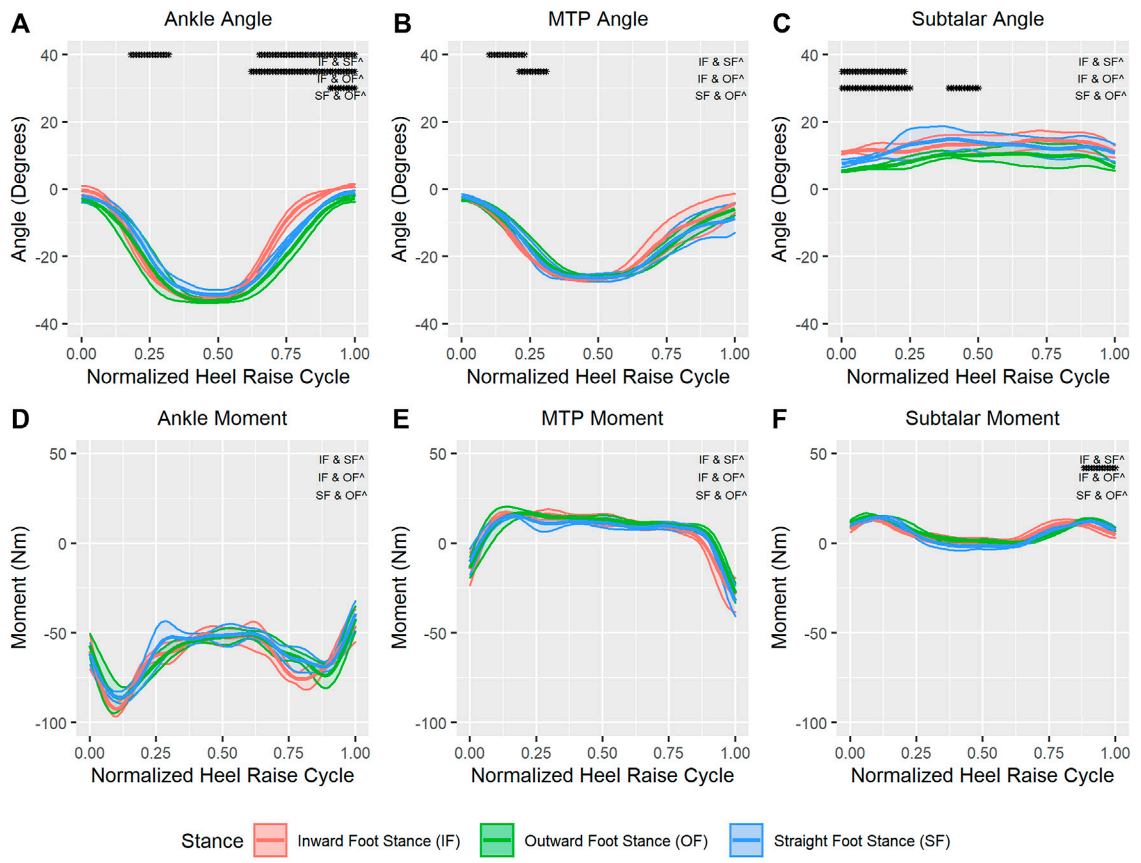

FIGURE 5
Average (±SD) ankle joint angle **(A)** and moment **(D)**, MTP joint angle **(B)** and moment **(E)**, and subtalar joint angle **(C)** and moment **(F)** for outward, inward, and straight foot stances through a normalized heel raise cycle. Solid center lines indicate means, and the surrounding shaded areas indicate ±1 standard deviation. Asterisks indicate statistical significance (α = 0.05) through a phase of a minimum of 10 consecutive statistically significant time normalized points.

significantly different phases. In particular, the inward and straight stances for the subtalar joint had different moments during the end phase of the heel raise cycle. Like the kinematic curves, this can be contributed to the choice of the end-of-cycle time point. Finally, change in stance had very little impact on the location of the center of pressure centroid relative to the stance of the feet. The three stances with the HULK and straight stance without HULK share visually similar hysteresis paths for their center of pressures.

As can be predicted, there were significant differences between the kinematics and dynamics of straight stance heel raises with and without the HULK device. The most prominent difference in the kinematics appears in subtalar joint at the peak of the heel raise. The straight stance without HULK repetitions displayed much less inversion than with the HULK. It is likely that the additional force the HULK device applies to the lower body requires greater recruitment from the muscles, which alters the kinematics of the exercise. This claim is supported by the large change in moment in the ankle and subtalar joint. The ankle, for example, had a larger resistive moment throughout the entire movement to maintain stability at higher loads, which will inevitably require larger muscle activations. Interestingly, the subtalar joint moment remains relatively constant throughout the heel raise cycle in the condition without the HULK, while the subtalar moment with the HULK generates a sinusoidal-like curve. This is certainly a consequence of the higher load and the change in kinematic angles that arise from this additional load.

Future spaceflight missions will inevitably include heel raises as an exercise in the astronaut's routine. If equipment similar to the HULK device is available, our results indicate that the stance of the astronaut will most likely not have any significant impact on the kinematics or dynamics of their HR lift. This information could be useful if certain joint angles or moment limits are trying to be avoided to minimized injury risk. These results indicate that users should select a stance that is the most comfortable and/or targets a desired lower-body muscle group. For example, studies used EMGs to identify that the medial gastrocnemius activates more than the lateral gastrocnemius when the subject performs the exercise with an outward foot stance (Riemann et al., 2011; Cibulka et al., 2017). When an inward foot stance is used, the lateral gastrocnemius activates more than the medial

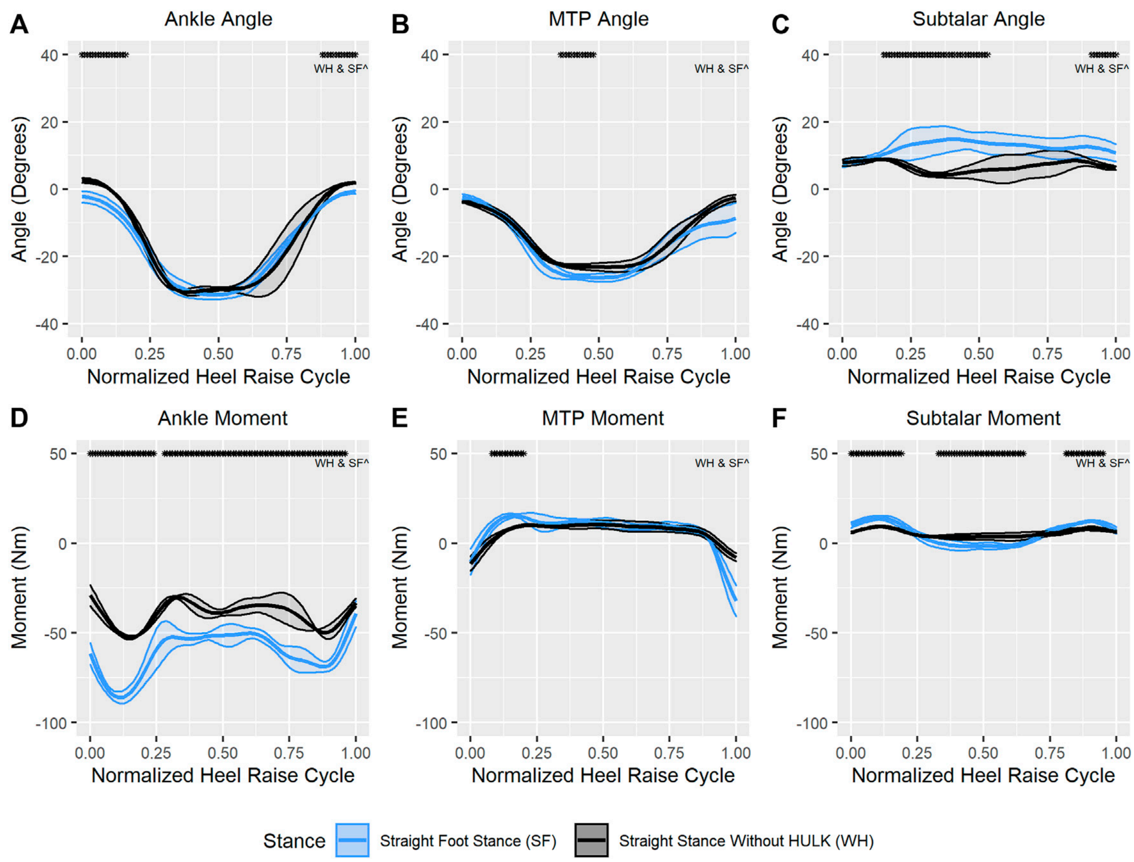

FIGURE 6
Average (±SD) ankle joint angle **(A)** and moment **(D)**, MTP joint angle **(B)** and moment **(E)**, and subtalar joint angle **(C)** and moment **(F)** for straight foot stance and straight stance without HULK conditions through a normalized heel raise cycle. Solid center lines indicate means, and the surrounding shaded areas indicate ±1 standard deviation. Asterisks indicate statistical significance (α = 0.05) through a phase of a minimum of 10 consecutive statistically significant time normalized points.

gastrocnemius. Also, when the subject performs heel raises with increased flexion in the knee, there are increases in activation of the soleus muscle with respect to the medial and lateral gastrocnemius muscles (Signorile et al., 2002). With known activation of the soleus and medial and lateral gastrocnemius, a workout program can be designed to target the desired muscle or muscle groups that are most affected by microgravity by altering foot stance. A combination of targeted muscle activation and proper muscle loading will allow for improvements in muscle mass in the lower leg muscles. The choice of foot stance will be one part of a comprehensive workout program to mitigate the loss of gastrocnemius and soleus muscle mass. In summary, the change in foot position during heel raises on the HULK device does not affect the kinematics and dynamics but based on previous studies (Riemann et al., 2011; Cibulka et al., 2017), we hypothesize that it does affect the activation of the gastrocnemius and soleus. This can be coupled with proper loading and exercise programs to create an effective spaceflight-induced muscle loss countermeasure. Finally, this research is applicable and informative for Earth-based exercise and rehabilitation. Like astronaut exercise regimes, lower body rehabilitation plans frequently incorporate heel raise exercises to strengthen muscle groups in the lower legs. Depending on the injury, certain foot stances during the exercise may be more desirable to concentrate on certain muscle groups or to avoid others. Thus, while muscle activation can be different, the kinematics and moments affecting the joints in the foot will remain constant regardless of stance. In addition, for both Earth-based and space-based exercise devices, it is important to build in flexibility that allows the subjects and workout program creators the opportunity to alter standard exercises to target specific muscles groups by altering foot stance when performing heel raises and squats.

Limitations and future work

Our study has several limitations. First, the study utilized only one subject for a limited number of repetitions, which limits the power and generality of our conclusions. Future work should expand to more subjects completing a higher number of

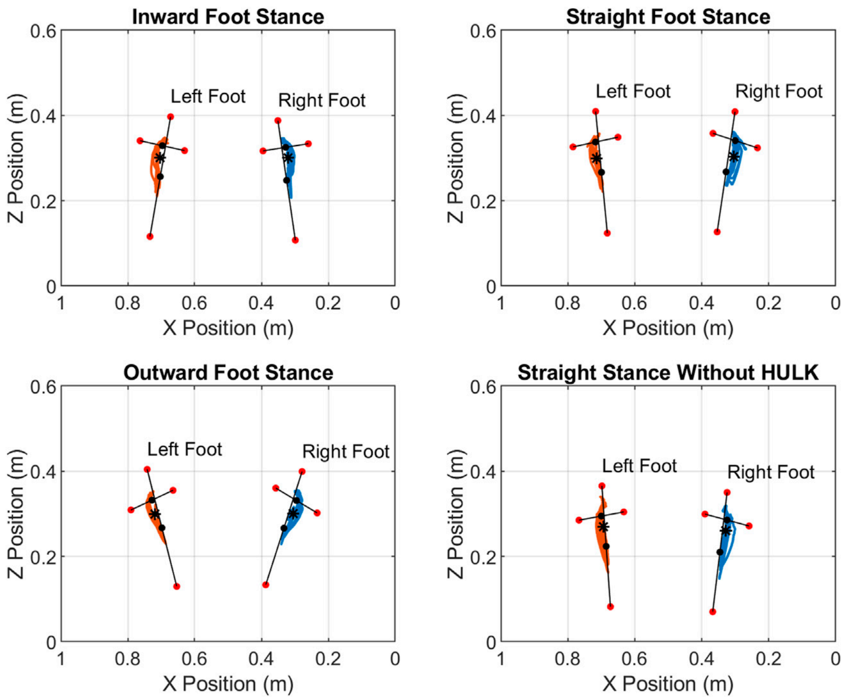

FIGURE 7
Center of Pressure during the heel raise repetitions for the four different configurations investigated: inward, straight, and outward foot stances with the HULK device, and straight stance without the HULK device. The blue line indicates the center of pressure of the right foot, and the orange line indicates the center of pressure of the left foot. Red dots indicate the location of motion capture markers on the toe, heel, outside, and inside of the left and right feet. Black dots indicate center points of the toe/heel line and the inside/outside line. The asterisks represent the centroid of the center of pressure through the entire set of HR repetitions. Note: the x axis has its origin on the right side of the figures and increases towards the left. This is due to the origin of the force plates being located in the bottom right corner.

repetitions. In addition, future studies could also include a larger range of stance angles to assess and identify the dose response curve of foot stance to heel raise kinematics and dynamics. While our results indicated small variations in kinematics and dynamics when using different foot stances, additional study of muscle activation, and specifically the impact of the HULK device to muscle activation, will be desirable and advantageous to further characterize the performance of the HULK device. Finally, heel raises on the HULK device are performed with the load pulling the subject in front of the body and at the waist. The effect of this location compared to the load located on the subject's back or in a seated position (e.g., on a leg press machine) will need to be addressed in future studies. Despite these limitations, the results of this study create a foundation that simultaneously supports future work and brings new insights into the development and applications of exercise protocols and countermeasures on Earth and in space.

Data availability statement

The raw data supporting the conclusion 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 NASA Institutional Review Board. The patients/participants provided their written informed consent to participate in this study.

Author contributions

LK, RP, WT, BL, and AD-A all contributed to the creation and analysis of the data. In addition, each contributed to the writing and editing of this 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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Appendix

TABLE A1 Average (±SD) peak angle, range of motion, peak moment, and angular impulse during heel raises for inward foot stance, outward foot stance, straight foot stance, and straight stance without HULK conditions.

Joint	Stance	Peak angle (degrees)	Range of motion (degrees)	Peak moment (N*m)	Angular impulse (N × m × s)
Ankle	Inward foot stance	−32.8 (±0.7)	33.8 (±0.8)[b,c]	−92.5 (±4.3)	−68.1 (±6.0)
	Outward foot stance	−33.3 (±0.5)	30.8 (±0.9)[a]	−88.6 (±3.8)	−71.1 (±4.8)
	Straight foot stance	−31.7 (±1.2)	30.8 (±0.9)[a]	−86.4 (±3.2)	−65.3 (±4.1)
	Straight without HULK	−30.9 (±0.9)	33.4 (±0.2)[d]	−52.7 (±0.9)[d]	−43.4 (±4.7)[d]
MTP	Inward foot stance	−26.9 (±0.6)	24.7 (±1.0)	17.2 (±1.4)	8.2 (±0.5)[b]
	Outward foot stance	−26.2 (±0.6)	23.3 (±0.4)[c]	17.4 (±1.4)	10.2 (±1.1)[a,c]
	Straight foot stance	−26.8 (±0.4)	24.6 (±0.7)[b]	15.9 (±1.0)	7.8 (±1.1)[b]
	Straight without HULK	−23.4 (±1.3)[d]	20.9 (±0.4)[d]	10.9 (±1.8)[d]	7.2 (±2.3)
Subtalar	Inward foot stance	15.1 (±2.2)	5.4 (±1.2)	14.0 (±0.5)	6.3 (±0.9)
	Outward foot stance	12.5 (±2.0)	7.3 (±2.0)	15.8 (±0.9)	7.4 (±0.5)[c]
	Straight foot stance	16.4 (±3.0)	8.8 (±3.5)	14.5 (±0.9)	5.8 (±0.9)[b]
	Straight without HULK	9.8 (±1.5)[d]	6.0 (±0.9)	9.3 (±0.6)[d]	6.1 (±1.0)

[a]Statistical difference from inward foot stance (α = 0.05/3).
[b]Statistical difference from outward foot stance (α = 0.05/3).
[c]Statistical difference from straight foot stance (α = 0.05/3).
[d]Statistical difference between the straight foot stance and straight stance without HULK, condition (α = 0.05).

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The application of artificial gravity in medicine and space

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Gravity plays a crucial role in physiology. The lack of gravity, like in long duration spaceflight missions, cause pathologies in *e.g.*, the musculoskeletal system, cardiovascular deconditioning, immune system deprivation or brain abnormalities, to just mention a few. The application of artificial gravity through short-arm human centrifugation (SAHC) has been studied as a possible countermeasure to treat spaceflight deconditioning. However, hypergravity protocols applied by using SAHC have also been used to treat different, ground-based pathologies. Such gravitational therapies have been applied in Uruguay for more than four decades now. The aim of this overview is to summarize the most important findings about the effects of gravitational therapy in different, mainly vascular based pathologies according to the experience in the Gravitational Therapy Center and to discuss the current research in the field of hypergravity applications in medicine but also as multisystem countermeasure for near weightlessness pathologies. New insight is needed on the use of hypergravity in medicine and space research and application.

KEYWORDS

human centrifugation, microgravity, Peripheral Artery Disease (PAD), Coronary Artery Disease (CAD), Lymphedema, Complex Regional Pain Syndrome (CRPS), Secondary Raynaud's Phenomenon, Systemic Sclerosis

1 Introduction

Gravity (g) plays a vital role in physiology. Near weightlessness in long duration spaceflight, causes pathologies such as severe loss of bone density and skeletal muscle strength, cardiovascular deconditioning, immune system deprivation, changes in brain morphology, reduced cognition or changes in vision (Paez et al., 2020; Roberts et al., 2017; Stepanek et al., 2019). Increased gravity (hyper-g) on the other hand is experienced by

Abbreviations: AG, artificial gravity; CRPS, complex regional pain syndrome; CAD, coronary artery disease, ECs, endothelial cells; GT, gravitational therapy, GTC, gravitational therapy center; HDBR, head-down tilt bed rest; HV, healthy volunteers; PAD, peripheral artery disease, RP, Raynaud´s phenomenon; SAHC, short-arm human centrifugation; SSc, systemic sclerosis.

pilots of high-performance aircraft who are trained to undergo short periods of 8–9 g using dedicated training centrifuges. Centrifuges generating artificial gravity (AG) have been and still are being explored as multi-system countermeasure/ therapeutic devices for the earlier mentioned spaceflight related pathologies for future long-duration spaceflights. However, AG exposure through SAHC has been used as a therapeutic procedure called Gravitational Therapy (GT) in Uruguay for more than 40 years now. It has been applied for the treatment of different vascular based pathologies such as peripheral obstructive arteriopathies, coronary artery disease, lymphedema, Raynaud phenomenon, among others with very successful results (Isasi et al., 1986a; Isasi et al., 1986b; Isasi et al., 1990a; Isasi et al., 2001). Besides, SAHC was also used as a treatment for obstructive peripheral arteriopathies in a Russian facility at Samara State University (Makarov and Lukashou, 2018) and more recently as a promising physical rehabilitation approach for other medical conditions (Kourtidou-Papadeli et al., 2021a; Kourtidou-Papadeli et al., 2021b). The aim of this overview is to summarize the most important findings about the therapeutic effects of GT through SAHC in different vascular based pathologies mainly according to the experience in the Gravitational Therapy Center in Uruguay and to discuss the current research in the field of AG applications in space research.

2 Hypergravity in medicine and space flight

It was the Dutch mathematician Christiaan Huygens who first introduced the term "centrifugal force" in his 1659 work "*De Vi Centrifuga*". In human research and application, one of the very early predecessors of the current short arm centrifuges was probably the Cox's chair described more than two centuries ago in his book "*Practical Observations on Insanity*" (Wade, 2005). Also the later work by Halloran and his application of a circulating swing used in clinical medicine in treatment of mental health issues laid the ground works for rotating devices in human medicine (Breathnach, 2010).

2.1 History of gravitational therapy in Uruguay

In late 1940s cardiologists Dr. E.J. Isasi and Dr. R. Velasco Lombardini built a first human centrifuge in Uruguay with the aim to explore a possible physical procedure to reduce arterial hypertension. This application of AG was inspired by works emerging from the Royal Canadian Air Force in Toronto published by Franks, Kerr & Rose (Franks et al., 1945a; Franks et al., 1945b). At that period in time there was no medication to treat this condition, as it was before the widespread use of *Rauwolfia* derivatives, vasodilators (hydralazine) and peripheral sympathetic inhibitors (guanethidine) (see review (Moser, 2006)). Some preliminary successful results on patients with arterial hypertension and peripheral arteriopathy disease (PAD) were communicated in national scientific meetings in the mid-1950s (Velasco-Lombardini and Isasi, 1951). The centrifuge was no longer used until mid-1970s when cardiologists Dr. M.E. Isasi and Dr. E.S. Isasi began to explore the effects of AG in different cardiovascular pathologies. In 1979 they devised a new centrifuge that was later patented in Uruguay, Argentina and United States (Figure 1A). With this new system they founded the Gravitational Therapy Center (GTC) in Montevideo, Uruguay, in that same year. To date the GTC still receives patients referred from different medical institutions for the treatment of various vascular based pathologies.

2.2 GT protocol

M.E. Isasi and E.S. Isasi have developed hypergravity protocols in which subjects are placed on a human centrifuge (US patent 4,890,629) in supine position with the head towards the axis of rotation (Figure 1A) and are exposed to accelerative and decelerative profiles (+Gz) from 0 to a maximum of 6 g at foot level with a rapid onset to the peak of acceleration and a rapid ramp back (Figure 1B) (Isasi et al., 2001; Isasi et al., 1986a; Isasi et al., 1986b; Isasi et al., 1992a; Isasi et al., 1992b; Isasi and Isasi, 1992; Isasi et al., 1992c; Isasi and Isasi, 2007; Isasi et al., 2007; Isasi et al., 2014).

By this procedure a gradient of g levels is imposed from head to feet (Figure 1C) (Isasi et al., 2014) although in the very first centrifuge and protocols patients were exposed seated, as was based on aerospace pilot centrifuge protocols. In some protocols g profiles could reach up to 6 g at foot level during seconds in trained patients. Nowadays, most protocols applied use g profiles between 1.5 and ~2.5 g at foot level during longer periods in a total of 1-h treatment. These lower g levels are very well tolerated in most patients of a broad age range from both sexes and with beneficial therapeutic effects (Figure 1B). After some training sessions, patients receive in average 1-h session, 1–3 times/week to complete at least 20 sessions. Importantly, the number of sessions and the maximum g level reached may vary according to each patient condition (*e.g.*: age, medical condition, tolerance) (Isasi et al., 1990a; Isasi et al., 2007; Isasi et al., 1992a; Isasi et al., 1992b; Isasi and Isasi, 1992).

By means of safety belts, patients are strapped with their head close to the center of a nearly 2 m-radius centrifuge. Patients are provided with an eye cover and they are asked to avoid moving their heads during centrifugation to avoid Coriolis effects. The clinical assessment and arterial blood pressure measurement is always performed before and after GT protocol. Currently, ambulatory electrocardiogram and blood pressure monitoring during centrifugation are also used. A physician and assistant are

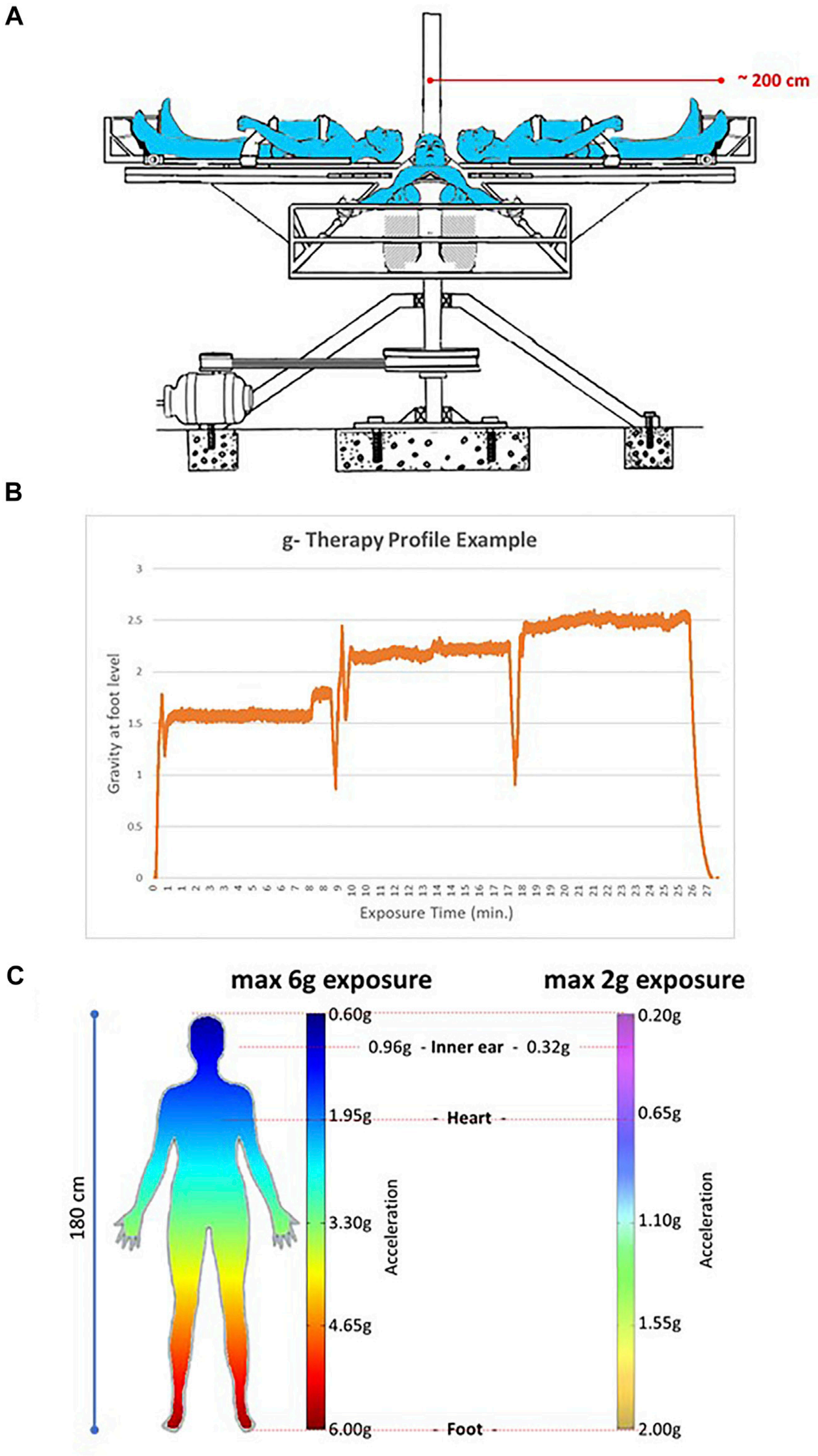

FIGURE 1
Centrifuge as currently used at the Gravity Therapy Center (GTC) in Montevideo, Uruguay. **(A)** Configuration of the nearly 2-m diameter centrifuge used for GT sessions at the GTC. A maximum of 4 patients can be accommodated in one session. **(B)** An example of a g profile used nowadays. The profile might be adapted based on patient's tolerance, medical condition or age. **(C)** Gravity gradient over the patient's 180 cm tall body when exposed to a maximum of 6 g or 2 g in a 2-m diameter system. Note that for the 6 g system the g at heart level is ~1.95 g and at the level of the inner ear/vestibular system nearly 0.96 g. For the 2 g this is 0.65 and 0.32, respectively.

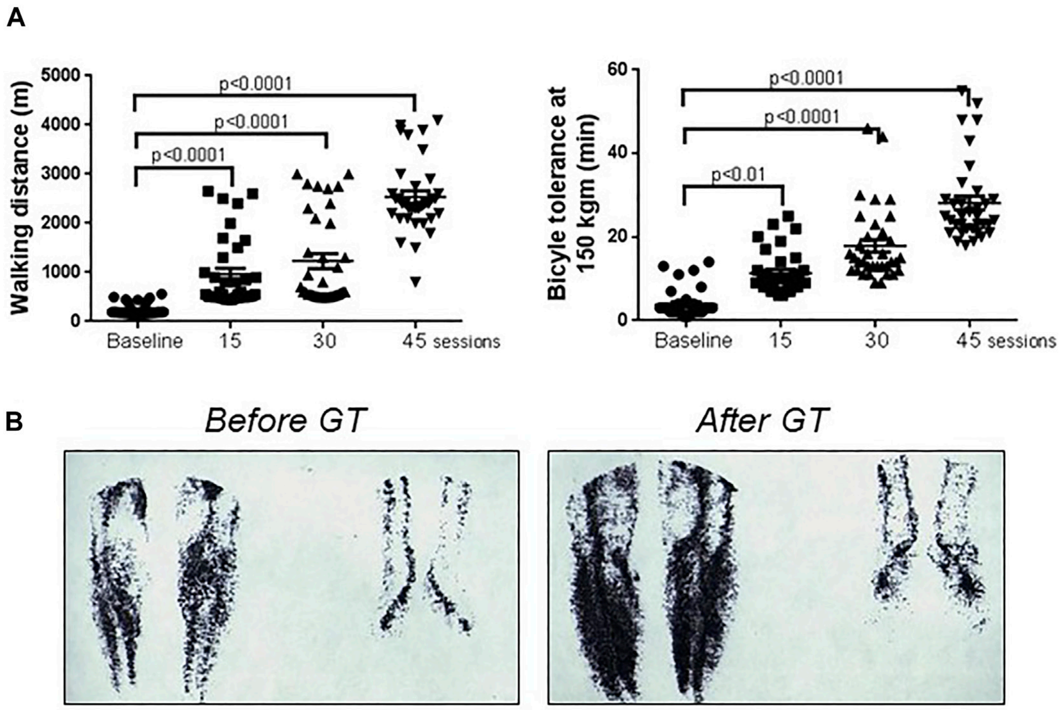

FIGURE 2
Evolution of patients with peripheral artery disease with gravitational therapy. **(A)** Functional recovery of 35 PAD patients with intermittent claudication (Fontaine stage II) evaluated through total walking distance (left chart) and bicycle tolerance at 150 kgm/min (right chart) following GT sessions. Note the significant improvement in both parameters evidencing functional recovery following GT sessions. **(B)** Static radionuclide angiography of calves and feet of a 57-year-old male patient with PAD before (left image) and after GT rehabilitation (45 sessions) (right image). Note the increase of blood circulation in the lower limbs. *Adapted from* (Isasi et al., 1990a).

always monitoring patients during centrifugation in case any patient requests to stop centrifugation by raising the hand or speaking out loud. Over more than 40 years, GT has demonstrated to be a safe and well tolerated therapeutic procedure, without significant side effects in the conditions applied. There were only sporadic transitory states of dizziness or nausea observed when the patient is not yet used to the procedure, so, the training of patients is important for better tolerance and continuity of the treatment.

2.3 Decades of studies on the effects of centrifugation on different pathologies

2.3.1 Peripheral artery disease (PAD)

By continuing the initial observations on the effects of centrifugation on PAD patients a study to rehabilitate patients with peripheral obstructive arteriopathies was initiated. 35 patients with intermittent claudication (Fontaine stage II) were exposed to hypergravity protocol during 30 min, 3 times/week during 20 weeks. Functional recovery was measured by the total walking distance and the duration of bicycle tolerance at 150 kgm/min at baseline and after 15, 30 and 45 hypergravity sessions (Isasi et al., 1986a). A very significant improvement in functional capacity was observed with the greater number of hypergravity sessions (Figure 2A). Interestingly, static radionuclide angiography of thighs and calves performed before and at the end of the study revealed a greater blood circulation in the lower limbs of PAD patients which correlated with the functional recovery (Figure 2B). In another study of 10 male patients with PAD, a daily protocol of GT was applied during 30 min along 4 weeks. A significant increase in walking distance, sural triceps test score and reduced ischemic pain was observed after 4 weeks (Isasi et al., 1990b). Thus, GT improved PAD patients by abolishing muscle ischemic pain and recovering the functional capacity due to an increase in the collateral circulation (Isasi et al., 1986a; Isasi et al., 1990b).

2.3.2 Coronary artery disease (CAD)

GT has also been studied in patients with CAD since mid-1970s, showing a significant improvement in functional capacity and in exercise tolerance as well as reduced angina pectoris (Isasi et al., 2000a). In a study of 20 patients with CAD, with mild or moderate hypertension, with or without angina pectoris and with

ST depression >0.2 mV, exercise bicycle testing was assessed before and after one GT session. The treatment resulted in a significant decrease in ST depression and increase in exercise duration without angina pectoris (Isasi et al., 2000a). This acute effect following one hypergravity exposure might indicate increased myocardial perfusion. In another study of 30 patients with proven CAD and 10 healthy volunteers (HV), endothelial cells (ECs) were counted in venous blood smears stained with May-Grünwald–Giemsa over 100 white blood cells (WBC) obtained before and after only one GT session. Very low or undetectable ECs were observed in the control group at baseline and after one hypergravity protocol (ECs/100 WBC: from 0.4 ± 0.5 to 0.7 ± 0.6). In turn, CAD patients showed a significant increase in ECs (ECs/100 WBC: from 4.27 ± 4.09 to 11.5 ± 7.55) (Isasi et al., 1998a). In addition, CAD patients did also show a statistically very significant increase in ECs number with respect to HV at baseline which is consistent with the literature that show increased number of circulating ECs associated with cardiovascular disease and its risk factors, such as unstable angina, acute myocardial infarction, stroke, diabetes mellitus, critical limb ischemia and related diseases (Boos et al., 2006; Deb-Chatterji et al., 2020; Schmidt et al., 2015). Thus, taken the results on PAD and CAD patients, it is feasible that GT would induce sustained vasodilation, increase endothelium turnover and collateral circulation.

2.3.3 Lymphedema

In the 1980s the GTC began a study on the possible effect of centrifugation on lymphedema patients (Isasi et al., 1986b). Lymphedema is a progressive pathological condition of the lymphatic system that involves the accumulation of protein-rich fluid, inflammation, swelling and subsequent induration and fibrosis that cause disfigurement of the affected region, decreased mobility and function (for review see (Warren et al., 2007) and (O'Donnell et al., 2020)). In a study of 30 patients with primary or secondary lymphedema due to surgical procedures, irradiation therapy, erysipelas infection or chronic venous insufficiency affecting only one lower limb (26 pts, 87%) or both lower limbs (4 pts, 13%) and 10 HV received a subcutaneous injection of 1–1.5 mCi of 99mTc Sb2S3 colloid into the foot first interdigital space. Gamma camera images (5 min-static images) were taken from the injection site and the abdomen immediately after and at 1, 2, 3 or 4 hs post-radionuclide injection in patients in supine position (Isasi et al., 1992a). In 1 g control conditions, the radionuclide liver uptake occurred at 3 hs in 10 (100%) HV and 26 (87%) lymphedema patients and at 4 hs in 4 (13%) lymphedema patients (Isasi et al., 1992a). In a second session using the same protocol but now including a hypergravity protocol (accelerative and decelerative profiles, 0–6, + Gz) exposure of 20 min, showed that in 10 (100%) HV and 28 (93%) lymphedema patients radionuclide liver uptake was observed already at 1h, in 1 (3%) patient at 2hs and in 1 (3%) patient at 3hs after tracer injection (Isasi et al., 1986b). Interestingly, in another study of 20 patients (3M, 17F, mean age 50 yrs old) and 10 HV (3M, 7F, mean age space, 48 yrs) two lymphoscintigraphic studies were performed at baseline and after centrifugation, without medication or after taking cyclooxygenase (COX) inhibitors (aspirin 500mg/8hs, indomethacin 25 mg/8 hs) starting 48hs prior to the study. Results showed that COX inhibitors delayed (2–4+ hs) the radionuclide liver uptake significantly in HV and lymphedema patients while without medication the liver uptake was observed at 1 h in 10 HV and 18 patients and 2 h+ in 2 patients after centrifugation (Isasi et al., 1990a; Isasi et al., 1992c). Thus, the hypergravity protocol applied constitutes a mechanical stimulus that accelerated the lymphatic drainage and the liver uptake of tracer in HV and patients and involved a mechanism related to, at least in part, prostaglandin synthesis (Isasi et al., 1990a; Isasi et al., 1992c). The relationship between endothelium derived prostanoids and the modulation of lymphatic tone, flow resistance, and lymphatic function has been documented in other papers (Gashev and Zawieja, 2010; Koller et al., 1999).

Patients with Primary or Secondary Lymphedema treated with GT showed reduced edema, improved mobility, reduced the frequency of erysipelas episodes, improved the quality of the skin and the sensitivity of the lymphedematous limbs (Isasi et al., 2001; Isasi and Isasi, 2007) (Figure 3). In a study that recorded the evolution of 275 lymphedematous limbs from 161 patients (138 F, mean age 50.6 ± 18 yrs and 23 M, mean age 42 ± 20 yrs) suffering from Primary or Secondary Lymphedema that were treated with an average of 20 GT sessions (two to three sessions/week) and a maintenance therapy of a minimum of 10 sessions annually, showed that 195 pts (71%) reduced more than 50% of the lymphedematous limb volume, 56 pts (20%) between 30–50% of the volume and 24 pts (9%) less than 30% of the limb volume (Isasi and Isasi, 2007). After GT, adequate elastic support maintained the successful results. Although lymphedema cannot be completely cured, GT has demonstrated to significantly reduce edema and functional disability of the limb, improving the quality of life of these patients.

2.3.4 Complex regional pain syndrome (CRPS)

GT also has beneficial therapeutic effects in patients suffering from Complex Regional Pain Syndrome (CRPS) (Isasi et al., 1990c; Isasi et al., 1999). CRPS type I, also formerly known as reflex sympathetic dystrophy (RSD), has been described as pain that a patient feels which is disproportionate to the inciting event and is associated with autonomic dysfunction, swelling, dystrophic skin changes, stiffness, functional impairment and eventual atrophy (Neumeister and Romanelli, 2020). This disease affects musculoskeletal, neural and vascular structures and may be presented as continuing pain, allodynia or hyperalgesia, changes in skin perfusion or abnormal sudomotor activity and also thermoregulatory dysfunction with distinct vascular dysregulation patterns (Neumeister and Romanelli, 2020). In fact, changes in vascular sensitivity to cold and circulating

FIGURE 3
A lymphedema patient with gravitational therapy. A 48-year-old female patient with a severe lymphedema affecting left lower limb after 13 years of numerous erysipelas episodes (left). After 5 weeks of initiating gravitational therapy (10 sessions) there was a significant reduced edema (right). She could wear trousers and could sleep without the limb elevated. The leg perimeter had reduced from 70 to 29 cm, the quality of the skin improved and she did not repeat erysipelas.

catecholamines may be responsible for vascular abnormalities and may be associated with an abnormal (site dependent) reflex pattern of sympathetic vasoconstrictor neurons due to thermoregulatory and emotional stimuli (Baron and Maier, 1996). In a study of 26 patients (5M, 21F, mean age 42 yrs old) suffering from RSD triggered by trauma (22 pts) or by surgery (4 pts), patients were exposed to centrifugation (+Gz, 0–6g, 1 h) daily. The number of sessions was dependent on patient's response (from 2 to 20 sessions). Assessment of patients was performed by clinical evaluation, digital photoplethysmography and static radionuclide bone images. In 25 patients (96%) there was a dramatic pain relief and improvement in vascular response. Radionuclide bone images persisted positive in spite of the clinical improvement and good vascular response (Isasi et al., 1990c). Importantly, GT seems to be an effective therapy in the acute but not in the chronic stage of CRPS I (Isasi et al., 1999).

2.3.5 Secondary Raynaud's phenomenon and systemic sclerosis

Secondary Raynaud´s Phenomenon (RP) is associated with Systemic Sclerosis (SSc) and it is a vasoconstrictive response associated to endothelial dysfunction that is present in approximately 95% of patients with SSc (Meier et al., 2012). SSc is a progressive autoimmune connective tissue disease characterized by vascular abnormalities and fibrosis of the skin, joints and internal organs (Cutolo et al., 2010; Denton and Khanna, 2017; Quinlivan et al., 2020). Given that microvascular damage and endothelial dysfunction underlies SSc and that GT has shown beneficial effects on vascular diseases by inducing sustained vasodilation through prostacyclin release and by increasing endothelium turnover (Isasi et al., 1998a), GT was postulated to be beneficial in SSc patients. In a study of 46 patients (6 F, 40 M, mean age 48 ± 14 yrs old) with SSc that received GT, 3 times a week during 6 weeks, patients showed increased pulse wave amplitude and very significant increased ECs in venous blood smears after GT. Also, SSc patients showed a very good clinical evolution by reducing ischemic pain, less frequent Raynaud´s attacks and by healing digital ulcers avoiding, in severe cases, the digital amputation (Figure 4) (Isasi and Isasi, 2004). In this study the follow-up period was 37 ± 14 months and all patients received between 10 and 20 hypergravity sessions prior to winter as a maintenance therapy. With respect to skin fibrosis, SSc patients

FIGURE 4
Two examples of patients with severe secondary Raynaud´s phenomenon treated with gravitational therapy. **(A)** A 36-year-old female patient with severe secondary Raynaud's Phenomenon. Before treatment, severe vasoconstriction (pallor and cyanosis) and puffy fingers (left image). After gravitational therapy patient showed a remarkable improvement in blood circulation of both hands (right image). **(B)** A 44-year-old female patient diagnosed with mixed connective tissue disease that presented a 10-days- history of painful fingertips ischemia and necrosis in both hands (left image). The ischemic pain was so severe that kept her awake all night long. After one week of gravitational therapy pain reduced dramatically and she did not take analgesics any longer. After 20 weeks of gravitational therapy the fingers were completely healed (right image).

showed a clear improvement after GT. In a former study with 20 SSc female patients that received GT, skin sclerosis was assessed by the % of skin involvement applying the "rule of nines" as in burns and by a clinical skin severity core (CSSS) rating the thickness of the skin from 0 to 3+ in 15 areas of the body. In this study, the mean skin scores at the entry versus the end of GT were 50.21 vs. 19.80 for % of skin involvement and 22.72 vs. 9.16 for CSSS, showing an improvement in skin fibrosis (Isasi et al., 1998b). In addition to the beneficial effects reported on RP, digital ischemic lesions and skin fibrosis of SSc patients (Isasi et al., 1998b; Isasi et al., 2000b; Isasi and Isasi, 2004), digestive symptoms, mouth opening, mild esophagitis and esophageal hypomotility, did also improved after GT (Isasi et al., 2006). In addition to SSc patients, patients with localized scleroderma (morphea) or linear scleroderma did also show a significant improvement with GT. Six patients with linear scleroderma of upper or lower limbs (4F, 2M, mean age 16.0 ± 7.8 yrs old) that received GT over 3 months, showed a significant improvement in skin score, reducing sclerosis, regaining muscle strength and mobility of the affected limb (Isasi et al., 2007). In a more recent work that communicated the evolution of 90 patient (76F, 14M, mean age 50 ± 14 yrs old) with RP and SSc that had digital ischemic lesions (150 fingers with digital ulcers and 53 with critical ischemia) recorded at the entrance of therapy, received GT 2–3 times/week during 6–8 weeks. After rehabilitation, most patients received a maintenance therapy of 10–20 sessions annually. Most patients (98%) with digital ischemic vascular pain achieved complete pain relief after two to three hypergravity sessions and could refrain from opioid analgesics. In addition, frequency of RP attacks and severity was reduced, improving skin color, temperature, sensitivity and mobility because of reduced edema in hands. At the beginning, 33 patients had 53 fingers with critical ischemia and after GT, 31 patients (94%) completely healed the ischemic lesions while 2 patients (6%) suffered distal digital amputation during treatment. No adverse effect occurred in any patient exposed to GT during the follow-up period of 4.56 ± 3.18 years (Isasi et al., 2014). This study showed that GT had significant beneficial effects preventing the use of aggressive treatment such as potent vasodilators, opioid analgesics and even hospitalization in cases with severe digital ischemic lesions. This mechanical/biophysical stimulation of the macro- and micro vasculature seems to be in line with a more recent study by Mitropoulos *et al.* (Mitropoulos et al., 2018) which suggest that 12 weeks twice a week of high intensity arm cranking training has the potential to improve the microvascular endothelial function in SSc patients. Also, our own work with ECs exposed to hypergravity (Szulcek et al., 2015) and work by others (Maier et al., 2015) show a direct effect of gravity on ECs.

2.4 Biological effects of GT

Applying a GT protocol showed a vasodilator effect assessed with digital photoplethysmography at 5, 15, 30 and 60 min after centrifugation in patients and HV and it was abrogated with the use of COX inhibitors such as aspirin (500 mg/8 hs) or indomethacin (25 mg/8 hs) starting 72 hs prior to centrifugation (Isasi and Isasi, 1992; Isasi and Isasi, 1996). These initial results obtained led to study levels of 6-keto prostaglandin F1 alpha, a prostacyclin active and stable metabolite, in venous blood samples of 30 patients with coronary artery disease (CAD) and 4 controls. In control subjects this resulted in a statistically significant increase from 241 ± 50 pg/ml to 535 ± 76 pg/ml and in CAD patients from 167 ± 43 to 359 ± 79 pg/ml after only one GT session of 1 h (Isasi et al., 1998a). This suggests that the hypergravity protocol would induce a vasodilator effect dependent on prostacyclin synthesis and release. In addition, GT would have other important biological effects considering the multiple properties of prostacyclin such as the inhibition of platelet aggregation or white blood cells adhesion (Moncada, 2006). On the other side, data from basic spaceflight related research suggest that SAHC providing mild hyper-g (1–2 +Gz) results in a gravitational-induced rise of transmural pressure leading to constriction of resistance vessels that contribute to the maintenance of the mean arterial pressure as well as microvascular blood pooling contributing to soft tissue capacitance in the lower extremities (Watenpaugh et al., 2004; Habazettl et al., 2016). Although this acute effect of vasoconstriction in the lower extremities occurs during the acceleration phase at + Gz exposure due to high transmural pressures, in the deceleration and re-adaptation phases, the microvascular flow is recovered and enhanced as was demonstrated by digital photoplethysmography at different times after centrifugation (Isasi and Isasi, 1996). Importantly, given that the endothelium lining small and large blood vessels is an important tissue sensitive to *e.g.* shear stress/mechanical stimulations during the hyper-gravity and the re-adaptation phases of the treatment, it has been speculated that ECs might play a central role in the therapeutic effects observed (Isasi et al., 1998a). In this sense, it is possible that enhanced hemodynamic forces generated by GT would induce the synthesis and release of other biologically active substances such as nitric oxide (Isasi et al., 1998a), the increased expression of growth factors and cytokines and the modulation of endothelial gene expression in analogy with shear stress effects on the endothelium (Ando and Yamamoto, 2009; Davies, 2009).

Other possible mechanisms involved might be the process of vasomotion and underlying processes in small arteries. Vasomotion is modulated by changes in transmural pressures in small blood vessels (Gustafsson, 1993) but also lymph vessels show vasomotion related to lymph-induced transmural pressure and flow rate (Helden and Zhao, 2000; von der Weid, 2001). On a more molecular level, possible modulations of the tyrosine phosphorylation pathway involved in cellular signaling in arteriolar myogenic constriction (Murphy et al., 2002) might be relevant in the effects of GT. It might even be speculated, since the latter is also mentioned to be related to SSc, that protein tyrosine kinases might play a role in GT since they are known players in SSc disease pathogenesis (Sacchetti and Bottini, 2017).

2.5 Ground-based applications of short-arm human centrifugation with therapeutic benefits

There have been specific applications using centrifuges for the treatment of medical issues in the past but most seemed not to have developed into a more elaborate use. A case report from 1966 mention the use of a centrifuge at the Mayo Clinic (United States) to treat a retinal detachment. The patient was placed in different positions on the centrifuge and exposed to g levels between 2 and 2.7 g (Neault et al., 1966). Apparently, the Mayo Clinic had a human centrifuge available in that period so it is to be expected that it was also used for other treatments.

In another case report the centrifuge at NASA Ames (Moffett Field, United States) was used to relocate a bullet fragment in the brain of a 63-year-old male assault victim into a fixed position. A procedure was applied where the patient was positioned mainly towards the center of rotation and was exposed to a g profile with a maximum load of 6 g and the acceleration acting along the patient Gx, Gy and Gz axis being 5.1, 2.6 and 0.9 g, respectively. The total procedure lasted 58 s (Pelligra et al., 1970).

There are also reports from the Samara State Medical University (Samara, Russia) on the successful treatment of patients with chronic lower limb ischemia stage II with the support of a short arm centrifuge (Figure 5A) (Galkin et al., 2003; Makarov, 2003; Makarov and Lukashou, 2018). Unfortunately, most of these reports have not been published in international journals.

Very recently a SAHC has been proposed as a robust countermeasure to treat deconditioning and seems to be a promising physical rehabilitation approach towards the prevention of musculoskeletal decrement due to confinement and inactivity. Studies from Kourtidou-Papadeli *et al.* (Kourtidou-Papadeli et al., 2021a; Kourtidou-Papadeli et al., 2021b) show significant changes in heart rate variability, cardiac output, cardiac index, cardiac power and mean arterial

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FIGURE 5
Some examples of short arm centrifuges used in treatment and in support of various space flight programs as countermeasure against microgravity related pathologies. **(A)** One-person gravity therapy centrifuge at the Samara State Medical University, Russia (Orlov and Koloteva, 2017). **(B)** Upgraded SAHC from European Space Agency, ESA, at the Olympic Sport Centre Planica, Slovenia (image ESA & Jozef Stefan Institute Slovenia/ ©K. Bidovec & A. Hodalič). **(C)** Centrifuge at: Department of Health Technology, Space Institute of Southern China, Shenzhen, Guangdong, China. **(D)** NASA short radius centrifuge currently being re-installed at Texas A&M University, United States (Arya et al., 2007). **(E)** Short arm centrifuge at the Institute for Biomedical Problems, IMBP, Moscow, Russia. **(F)** Human Centrifuge at the:envihab center of the German space agency, DLR near Cologne, Germany (Frett et al., 2014).

pressure at different AG levels as compared to standing position by using a protocol with a +Gz force up to 2 g at foot level. Interestingly, SAHC applied as a 4-weeks training program including three weekly sessions of 30 min of intermittent centrifugation at 1.5–2 g in a 54-year-old male patient with multiple sclerosis (MS) showed significant improvement in cardiovascular parameters, muscle oxygen consumption and MS-related mobility disability and balance capacity (Kourtidou-Papadeli et al., 2021b).

2.6 Artificial gravity in space flight

That the lack of gravity during orbital space flight might not be compatible with human physiology was already recognized by the Russian mathematics teacher and space pioneer Konstantin Tsiolkovsky (1857–1935). He was probably one of the first to perform serious engineering work on human space travel and he drew the concept of generating AG by rotating the complete spacecraft where humans could maintain a proper physiology, and thus survive, in outer space. This concept was elaborated upon by the Slovenian army officer Herman Potočnik published in 1929 (Noordung, 1929) and the well-known torus-shaped station from von Braun is also a large-radius rotation space station, published in 1952 (see for overview of space stations (Logsdon and Butler, 1985)).

Current research regarding AG and its application in long duration space missions is very much focused on relatively short-radius centrifuges. The dimensions of such short-arm human centrifuges are driven by the limited diameter of current spacecrafts' hull. See examples of such centrifuges in Figures 5B–F. In light of a possible multi-system countermeasure for microgravity related pathologies, numerous studies have been executed on such small systems. We will address the outcome of only a few of these findings:

The exposure of humans to a 5-days ground-based model for near weightlessness, −6° head-down tilt bed rest (HDBR), had a major impact on the heart function and geometry. A daily exposure of either 30 min continuous or 6 × 5 min intermitted at 1.0 + G_z at the heart in a 2.82 m radius centrifuge did not counteract the effects of simulated near weightlessness while chronic exposure to 1 g (being ambulatory) for 3 days after the HDBR protocol did reverse the effects (Caiani et al., 2014). Using the same AG protocol but now in a 60-days HDBR study, De Martino and colleagues wanted to explore the possible efficacy of AG to mitigate deterioration in standing balance and anticipatory postural adjustments of trunk muscles. All experimental groups had poorer balance performance in most of the parameters and delayed trunk muscle responses. However, there was mitigation of some aspects of postural control in intermittent 1.0 + G_z exposure (De Martino et al., 2021).

Muscle performance was explored in, again, the same AG protocol but now in a 5 days HDBR study and AG 1.0 g + G_z set at the subjects' estimated center of mass. The AG protocols were not able to prevent the catabolic effects of HDBR upon muscle and bone. There was, however, a preservation of vertical jump performance by AG but this was likely caused by central nervous rather than by peripheral musculoskeletal effects (Rittweger et al., 2015).

From a 21-days bedrest study where the treatment group was exposed to a continuous 1 h/day of AG (2.5 g at foot level, 2.2 m diameter centrifuge: +G_z at heart level ~0.97 g) it was concluded that AG had the potential to maintain the functional (torque-velocity relationships of the knee extensors and plantar flexors of the ankle), biochemical and structural (mRNA and histology of vastus lateralis and soleus muscles biopsies), homeostasis of skeletal muscle in the face of chronic unloading (Caiozzo et al., 2009).

Also, a 2 × 30 min/day AG could not prevent the decreases in exercise capacity after the 4 days HDBR in male subjects. The authors recognized some indications to reverse hypovolemia induced by bed rest while they also concluded that SAHC might eliminate the changes in autonomic cardiovascular control (Iwasaki et al., 2001).

AG of 1 g at the center of the mass for either 30 min continuous or 6 × 5 min/day during 5 days HDBR exposure showed no relevant changes in bone markers such as urine levels of collagen type I C-Telopeptide (CTX), N-Telopeptide (NTX) as well as deoxypyridinoline (DPD) or serum betaCTX concentrations. However, there were significant changes in sCD200 and sCD200R1 markers (Kos et al., 2014) although the latter molecules are not widely used as bone related markers.

Bed rest decreased plasma volume, supine and head up tilt stroke volume in both HDBR as well as AG groups (1 h, 1.0 + Gz at the heart/day). Although plasma volume was decreased in control and AG after bedrest, AG was able to mitigate effects of 3 weeks of simulated microgravity on orthostatic tolerance and aerobic power. They argue that positive effects of AG on peripheral vasculature and improved sympathetic responsiveness to orthostatic stress might be the underlying mechanisms (Stenger et al., 2012).

3 Discussion

Gravitational Therapy as applied in Uruguay over more than four decades now, has shown important therapeutic effects in different medical conditions and in a broad range of patients' ages. Patients from both sexes showed significant improvement in medical conditions, although there are reported differences in cardiovascular response upon + Gz exposure (Masatli et al., 2018). However, there is no study assessing differences in the therapeutic effectiveness between males and females with similar ages and medical conditions. Importantly, there were no significant side effects recorded even in patients that had a follow-up period longer than 20 years that received 10–20 GT sessions annually. The side effects encountered were very sporadic and were mostly transitory states of dizziness/nausea or very rare case of vomiting mostly related to head movements during centrifugation, ergo Coriolis effects. Also, among our exclusion criteria for GT are patients with active cancer, with advanced stages of dementia, with generalized infection or erysipelas infections, pregnant women, etc.

GT has shown a vasodilator effect through prostacyclin release, improving tissue perfusion and probably inducing collateral circulation, all resulting in functional improvement in CAD and PAD patients. It also significantly improved lymphedema patients by fostering lymphatic drainage, improving the quality of the skin, mobility and function of the lymphedematous limb. GT had also important beneficial effects in Secondary RP and SSc reducing frequency of RP´s attacks, reducing ischemic vascular pain, improving tissue perfusion and reducing skin fibrosis. It is also remarkable to note the improvement of patients in the acute phase of CRPS I. Although there is a variability in the evolution of each patient that mostly depends on the years of evolution and stage of the disease, other possible comorbidities and the pharmacological treatment received (type and dose of drugs), the success of GT treatment is also dependent on the frequency and number of sessions along time. Even though most of the experience in Uruguay is focused on vascular based pathologies and the majority of its effects might be explained on the mechanical stimulus over the blood vessels and the cardiovascular system, other body systems such as the immune and nervous system might also be involved in the clinical improvements observed.

Besides, the beneficial therapeutic effects of GT in several pathologies (*e.g.* secondary RP, vasculitis, SSc, *etc.*), usually surpasses the effects of medication and the importance of GT in non-curable pathologies (*e.g.* SSc, lymphedema, among others) with limited conventional medical treatment options is remarkable. Also, the use of GT could limit or even eliminate the use of some pharmaceutics with important side effects (Balakumar et al., 2015; Mladěnka et al., 2018) especially when such drugs have to be chronically administered.

One of the major limitations for GT widespread use application is the need of optimization and standardization of hypergravity protocols adapted to each patient and its medical condition. Moreover, the design of randomized clinical trials comparing the effects of GT versus conventional medical treatment (usually pharmacological) are also needed to better identify the specific potential of GT aside from other medical interventions.

Interestingly, new research on the application of SAHC in other human pathologies such as MS is emerging (Kourtidou-Papadeli et al., 2021a; Kourtidou-Papadeli et al., 2021b) and supports the long-standing observations in Uruguay regarding the beneficial therapeutic effects of GT in multiple pathologies. Besides, results on the Russian facility at the Samara State University in PAD patients (Makarov and Lukashou, 2018) also seem to be in line with results obtained in the GTC since 1980s. More research, clinical trials and publications are needed in this field to achieve an international validation of the hypergravity treatment and its widespread use.

Although the list of SAHC spaceflight related studies as discussed earlier is by far complete, it illustrated that there is a remarkable part of the protocols applied and parameters measured where AG does not resolve the HDBR related deteriorations but, at best, mitigate them. In this short overview of SAHC as multi-system countermeasure for spaceflight related immobilization we solely included studies/data using only AG as single modulation and not studies including additional stimuli such as ergometers (Iwase, 2005; Wang et al., 2011) or jumping protocols (Dreiner et al., 2020). The addition of other exercise means in addition to AG, already indicates that current short duration short-arm centrifuge protocols by themselves are not sufficiently effective to counteract HDBR related changes.

Regarding AG research applied for future long duration space flight; current short duration studies are mostly focused on relatively fast changing organ systems such as the cardiovascular system, sensory-motor system or muscles. Nearly no work has been done to explore the effects of short arm centrifuges on *e.g.,* bone, brain (morphology as well as function), vision or skin where the space related pathologies are especially more pronounced after long duration exposures to near weightlessness (Tronnier et al., 2008; Vico et al., 2017; Roberts et al., 2019; Van Ombergen et al., 2019; Paez et al., 2020).

Although the current SAHC-AG protocols are not or not sufficiently effective; what are the reasons and alternatives for insufficient AG protocols as microgravity countermeasure? This could be the very limited exposure times; some 30–60 min/day in many protocols. The effect of a response is often directly related to the amplitude and duration of the applied stimulus. With a 30 min 2 g at foot level exposure one only applies 1.6% of the daily g-dose at heart level or only 3.2% with a 1 h/day exposure. One might argue that replacing a deleted biological/physiological stimulus with only a few percent of its original magnitude might not be sufficient to restore a physiological state. Also, as shown in pre-clinical studies, the vestibular system, for long seen as 'only' having a balance supporting function, is involved in vestibulo-autonomic regulatory mechanisms targeting other organ systems such as the cardiovascular (Gotoh et al., 2003), musculoskeletal (Luxa et al., 2013; Morita et al., 2020), or the temperature regulating system (Fuller et al., 2002). With an AG exposure of 2 g at foot level in a 2-m diameter centrifuge the g-load to the vestibular system is only 0.32 g (see Figure 1C). With a 30 min/day exposure this generates a 0.7% stimulus compared to regular physiological conditions. It is plausible to presume that the central nervous system does not receive sufficient afferent signals from vestibular otoconia to perform its regulating function. However, we have to recognize also short-term high impact mechanical loads are sometimes sufficient to maintain proper physiology (Buckner et al., 2020; Lambert et al., 2020).

From the hypergravity therapies as developed and applied by Isasi and colleagues we know that it is very well possible to expose humans, patients, to g-profiles with a short duration maximum of 6 g in +Gz laying supine. Although microgravity countermeasure protocols are also of 30 min to 1 hour in duration, going shortly to higher peak g levels of 6 g might be more effective to prevent near weightlessness related pathologies.

In addition, the short-arm systems under study for space exploration producing a huge body g-gradient of more than 300% (Goswami et al., 2021) does not reflect the gravity exposure a body is exposed to on Earth. One might also explore the application of long-arm systems where the body g-gradient is far less.

The alternative for maintaining proper physiology is to provide chronic gravity during long duration space missions. This would be achieved generating AG by rotating the complete spacecraft, or a large part of it, as it was already proposed by Tsiolkovsky. The g-level would be continuous like on Earth (at 1 g) or, when shown by research, a lower g-level but sufficient to maintain a healthy physiology. In such a configuration the crew also does not loose valuable crew time since in a short-arm system the subject cannot perform regular tasks when spinning because of the physical restraints but also the work capacity and tolerance are reduced compared to centrifuges with larger diameters (Nyberg et al., 1966).

Although no concrete evidence is provided by the authors, the initial response to such a solution would be that it is far too expensive or far too difficult to implement large radius centrifugation as mentioned by some scientists (Pavy-Le Traon et al., 2007; Hargens et al., 2013). However, various engineering studies clearly show that it is very well possible to assemble a rotating spacecraft which would be only 10–20% more expensive compared to a non-rotating system (Joosten, 2007).

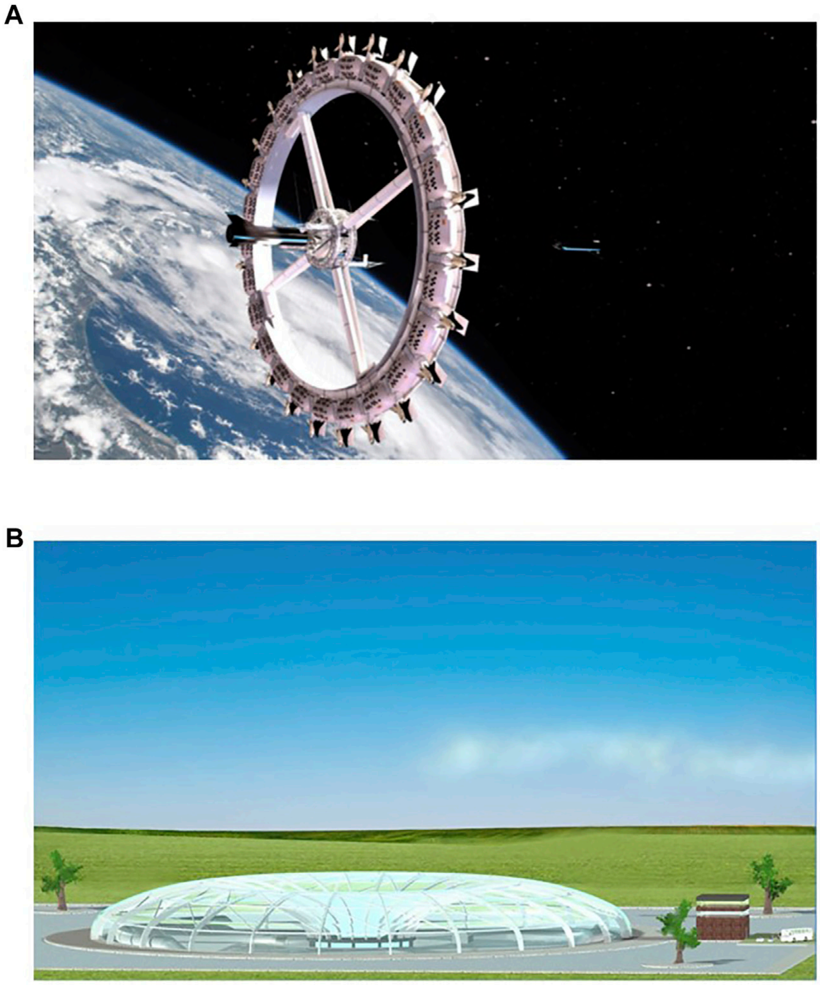

FIGURE 6
(A) The Voyager-class space station as proposed by Orbital Mechanics company (Fontana, CA, United States). This would be a ~200-m diameter continuous gravity (1/3 g) space station/space hotel planned for operation in 2027. (Image courtesy: Orbital Assembly, Huntsville, AL United States)) (Spilker, 2020). **(B)** A large artificial gravity platform; the Human Hypergravity Habitat, H^3; a proposed ground-based facility that could be used to obtain operational data regarding long duration/continuous rotation and moderate hyper-g on human physiology and psychology (van Loon et al., 2012).

Especially with the current significant drop in launch costs, this figure might even be reduced. Also, in terms of operation and spacecraft maneuvering flexibility, several recent studies have been published (Hall, 2016; Martin et al., 2016; Chen et al., 2020; Minster et al., 2020; Spilker, 2020; Dastjerdi et al., 2021) showing the feasibility of large rotating spacecraft. We should provide the crew, and later space tourists, with a physiological level of gravity the same as we do with providing a physiological level of oxygen or food. We need to provide gravity for proper health and reduce medical related mission risks. It would be unethical to deprive a human being from gravity (van Loon et al., 2020). More research should be devoted to this subject where a ground-based model for large diameter chronic rotation could be instrumental to develop the proper in-flight configuration (van Loon et al., 2012) (Figure 6B). Having such a large ground-based facility it could also be used to explore if this could be applied for treatments on *e.g.*, obesity and research regarding ageing. There are also commercial initiatives that already incorporate chronic AG in their on-orbit designs in order to provide a suitable/healthy environment for commercial customers (Figure 6A) (Spilker, 2020).

One might consider that the current suite of short-arm centrifuges developed for space-related countermeasure research could already very well be applied to also explore the use of hypergravity in various ground-based pathologies as a spin-off of the various human space programs.

In view of the great potentiality that the use of hypergravity could have to treat different medical conditions on Earth and as countermeasure to near weightlessness related pathologies, more research is necessary in this field. In this sense, it is urgently needed to establish international collaborations to foster this field of gravity research and applications in medicine. Importantly, more basic research is needed to unravel the biological mechanisms of human centrifugation and identify the cellular and molecular bases that explain the clinical improvement in patients exposed to this kind of therapy on ground but also for developing better AG protocols for long duration spaceflights.

Author contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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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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Multiple field tests on landing day: Early mobility may improve postural recovery following spaceflight

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Adaptation to microgravity causes astronauts to experience sensorimotor disturbances during return to Earth leading to functional difficulties. Recently, the Field Test (FT) study involving an incrementally demanding sensorimotor functional test battery has allowed for an unprecedented view into early decrements and recovery from multiple tests conducted on the landing day following 6-months International Space Station missions. Although the protocol was challenging and temporarily increased motion sickness symptoms, there were anecdotal reports that performing these tasks within the first few hours of landing accelerated their recovery. Therefore, results from computerized dynamic posturography (CDP) following return to Houston were used to compare recovery between crewmembers that participated in FT (n = 18) with those that did not (controls, n = 11). While there were significant decrements in postural performance for both groups, some FT participants tended to perform closer to their preflight baseline in the most challenging condition of the CDP sensitive to vestibular function—eyes closed, unstable support and head movements. However, the distribution of difference scores appeared bimodal with other FT participants in the lower range of performance. We attribute these observations to the manner in which the field tests were implemented—some benefitted by encouraging early movement to drive adaptation when performed in a constrained incremental fashion; however, movements above aversive thresholds may have impaired adaptation in others. Challenging the sensorimotor system with increasingly provocative movements performed as close to landing as possible, as long as within individual thresholds, could be a useful intervention to accelerate astronaut's sensorimotor readaptation that deserves further study.

KEYWORDS

vestibular, rehabilitation, posturography, sensorimotor, incremental

1 Introduction

Alterations in sensorimotor processing during spaceflight lead to performance decrements in functional tasks following transitions from microgravity to a gravitational environment. The greatest decrements in performance occur during functional tasks that require dynamic control of postural equilibrium (Miller et al., 2018; Mulavara et al., 2018). Exercise countermeasures available on the International Space Station (ISS) especially those conducted late inflight appear to improve recovery (Kozlovskaya et al., 2015; Loehr et al., 2015); however, competing constraints on exploration vehicles will limit future inflight countermeasures available (Chavers et al., 2021). Therefore, countermeasure strategies are needed to enhance sensorimotor adaptation to mitigate risks following landing on planetary surfaces where external support will not be available.

We propose that early mobility with incrementally increasing sensorimotor challenges, as long as movements are kept within one's motion tolerance, may optimize adaptation to the new gravitoinertial environment. This is based in part on evidence from cerebellar neurons that comparison of actual and predicted sensory feedback during voluntary self-motion appears to be critical in updating internal models associated with motor learning (e.g., Brooks et al., 2015). In particular, motor learning tasks that incorporate incremental error signals are more effective in driving neural plasticity and learning (Kagerer et al., 1997; Cakit et al., 2007; Schubert and Migliaccio, 2019). In addition to an incremental approach involving active movements, an early intervention following the G-transition may be equally important. Vestibular rehabilitation following acute peripheral loss appears to benefit from earlier exercises (Michel et al., 2020) in the same way that earlier mobility can improve rehabilitation outcomes in intensive and intermediate care settings (Drolet et al., 2013; Dirkes and Kozlowski, 2019).

Exercises with increasing levels of difficulty customized to an individual's state of recovery is consistent with our post-landing strategy (Wood et al., 2011). However, the supervised reconditioning program is typically delayed by more than 1 day while crewmembers return from the Soyuz landing site in Kazakhstan. Field Tests (FT) were conducted at the landing site to quantify functional postflight performance following long duration missions lasting ~6 months and track their recovery (Reschke et al., 2020). While not designed to be a rehabilitation-type study, the testing constraints followed similar guidelines as we propose. Participants performed a series of incrementally more difficult mobility-related tasks at both the landing site and the refueling stop during their direct return. Tasks were not completed when the motion would be considered above an aversive threshold (e.g., elicit vomiting). The intent to capture initial decrements as close to landing as possible ensured an earlier implementation of the protocol.

The purpose of this paper was to determine if these early, multiple testingon landing day improved postural recovery in the participating crewmembers compared to those who did not participate. Specifically, we compared measures between groups using Computerized Dynamic Posturography (CDP) measures conducted the day after landing (Wood et al., 2015). Based on the most challenging CDP test conditions requiring effective use of vestibular input (standing eyes closed on unstable surface with head erect or performing pitch head tilts), postflight postural recovery appeared improved in some field test participants versus non-participant controls. However, the bimodal nature of responses suggest that others may have pushed beyond their motion tolerance limit in an effort to complete more FT objectives. These observations are consistent with encouraging early movement to drive adaptation but performed in a constrained fashion to minimize movements above aversive thresholds.

2 Methods

2.1 Subjects and timeline

Twenty-nine United States Orbital Segment (USOS) astronauts returning from long-duration ISS missions in the 2010–2020 timeframe were included in our analysis. Eighteen subjects participated in the FT protocol described below, and data from 11 control astronauts who did not perform FT were obtained from NASA's medical data repository. FT participants include nine in a pilot FT (PFT) protocol and nine in the full protocol (full FT). Two of the control subjects overlap groups, with one participating in the PFT on an earlier mission and the other as a full FT participant in a later mission. For both of these crewmembers, their flights were several years apart. Although we expect some dependence between the same individual on different missions, these were included to maximize the subject pool per cohort. Specific expedition numbers or lengths of missions were not referenced to minimize the risk of data attributability according to NASA policy. While FT were conducted on both USOS astronauts and Russian cosmonauts (Reschke et al., 2020), we limited this analysis to USOS subjects who had multiple tests on landing day and early CDP data were available. The control group had similar male/female ratio, age range, flight experience, mission duration and timing for the postflight CDP test as the FT groups (Table 1).

Crewmembers were typically assisted out of the capsule and carried to the medical tents at the Soyuz landing site for assessments and field testing, and then assisted to helicopters to be flown to the nearby rally airport. Nominally, all subjects completed PFT or FT at the Soyuz landing site (Kazakh Steppe) in the medical tent within 1–2 h of landing. If the medical tent was not deployed at the landing site or tests could not be performed there, tests were performed at an airport 4–5 h after landing. This test session occurred an average of 2.32 ± 1.3 h after landing. All USOS crewmembers were then assisted to

TABLE 1 Demographics of the three cohorts, those who participated in Pilot Field Test, full Field Test, and Controls. Note that PFT and Full FT cohorts have been combined for final analyses.

Cohort	Subject count (male/Female)	Age (y, mean ± std)	Flight number (mean, range)	Mission duration (d, mean ± std)	Time of CDP tests (h, mean ± std)
Pilot FT	9 (8M, 1F)	46.7 ± 6.1	2.2, 1–4	179 ± 15	40.9 ± 5.8
Full FT	9 (8M, 1F)	50.2 ± 5.8	1.9, 1–4	167 ± 25	31.8 ± 9.0
Control	11 (10M, 1F)	48.2 ± 3.3	2.0, 1–3	159 ± 23	34.8 ± 4.3

the Gulfstream aircraft to be flown from Kazakhstan to Houston, TX. The aircraft is equipped with couches converted into beds to allow sleep and medical supplies to provide intravenous fluid therapy and medications as needed (Patlach and Alexander, 2013; Lee et al., 2020). One refueling stop in western Europe provided the opportunity for a second test session en route, on average 13.4 ± 0.8 h after landing. Both FT and no-FT participants typically ambulated with assistance during the refueling stop. Following the return flights, there was ambulation at Ellington Field and the astronaut crew quarters before transport to the testing facilities at the Johnson Space Center (JSC). The CDP session was then performed at JSC between 23–51 h (35.8 ± 6.9 h, mean ± std) after landing, with variations due to flight and testing constraints. All subjects gave informed consent according to the requirements of the Institutional Review Boards.

2.2 Field test

The specific Field Test protocol has been described elsewhere (Lee et al., 2020; Reschke et al., 2020). The common mobility tasks performed across both PFT and full FT protocols included sit-to-stand, recovery from fall (prone to stand) and tandem walk, performed in that order of increasing difficulty. For the sit-to-stand, crewmembers stood up without using their hands and remained standing for 10 s. The recovery from fall involved rising from a prone position and standing for up to 4 min. The tandem walk was the most challenging and performed last, requiring 10 heel-to-toe steps with arms crossed, and repeated with eyes closed and open. Full FT also included a timed up and go mobility test (sit-to-stand, walk 4 m, turn 180° and return to seated) with small obstacles (5–15 cm height) to step over on the return path (Reschke et al., 2020). Additional full FT tasks included a standing posture test with an upper body perturbation (push) and passive dynamic visual acuity during vertical linear oscillations on a spring-loaded chair. There were also a variety of seated tasks (eccentric gaze, dysmetria finger to nose, eye-hand coordination on a tablet, grip force discrimination) that were interspersed in the full FT testing protocol. If crewmembers were not comfortable performing the more difficult functional tasks, they were allowed to perform the seated tasks alone. Four of the 18 FT participants included in this analysis were not able to complete the full test battery due to motion sickness at the landing site and refueling airport. As stated above, stopping activity outside of one's motion threshold is a key feature of the incremental rehabilitation approach we are recommending.

2.3 Computerized dynamic posturography

CDP measures were conducted as part of medical assessments used to quantify the initial postflight decrements and recovery of postural stability (Wood et al., 2015). Multiple preflight CDP tests were conducted to minimize the effects of learning, and the preflight measurements used in this analysis were obtained from the last preflight session, usually 3 months before launch. CDP was conducted using a modified EquiTest system (NeuroCom International, Clackamas, OR). Subjects were instructed to maintain stable upright posture with arms folded across the chest. This early postflight session is limited to two Sensory Organization Test conditions with eyes closed, sway-referenced base of support, with three trials of head erect (SOT-5) followed by three trials of head moving (SOT-5M). The sway-referenced rotations of the support surface about the ankle joint are directly proportional to anterior-posterior (AP) sway to disrupt proprioceptive feedback. With this unstable platform and eyes closed, these conditions are the most sensitive to disruptions in vestibular processing and have the greatest diagnostic accuracy in detecting postflight decrements (Jain et al., 2010). Subjects wore noise-cancelling headphones through which operator instructions and white noise were supplied to mask external auditory orientation cues. Sinusoidal pitch head movements were paced at 0.33 Hz by an audible tone at ±20° guided by operator using feedback from a motion tracker mounted to the headphones (MTx, Xsens Technologies, Netherlands).

The AP peak-to-peak sway angle was used to compute a continuous equilibrium (cEQ) score between 0 and 100 that factors in the time before a fall occurs, thus separating ballistic falls from falls that occurred later in the trial (Wood et al., 2012).

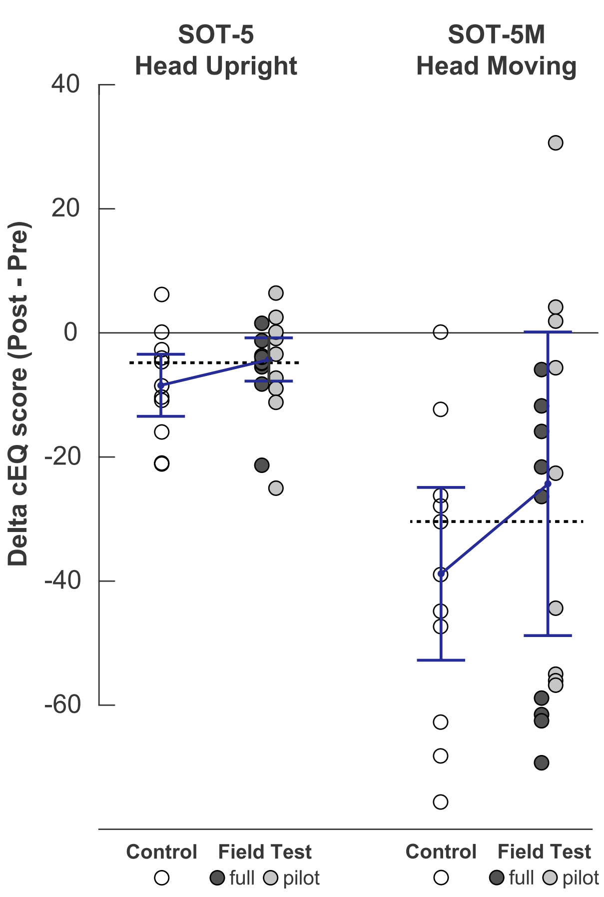

FIGURE 1
Comparison of postflight postural performance in FT participants and controls. The left panel is the SOT 5 condition (eyes closed, sway-reference support, with head upright). The right panel is the SOT-5M condition (eyes closed, sway-referenced support, with pitch head movements). Individual data points represent the difference between pre- and post-flight Equilibrium scores, with medians ± IQR for each group overlaid. For reference, the dashed line represents the grand median for all groups.

Falls were marked when subjects moved their feet, began to take a step, or raised their arms. The median cEQ score of the three trials were calculated for both SOT conditions, and the delta cEQ scores were computed (Post-Pre) with higher numbers representing better performance. Goodness of fit to normal distributions were evaluated with Shipiro-Wilk statistic. Due to the skewed nature of the cEQ scores, non-parametric Wilcoxon signed-rank test were used for comparing paired pre-to-postflight differences, the Mann-Whitney test for comparing FT and control independent groups, and Spearman Rank correlation (r_s) for examining strength of relationships. Based on the Mann-Whitney test statistic, we calculate the probability of superiority (PS), or the probability of an observation in the FT group having a true value that is higher than an observation in the non-FT group, as a measure of effect size (Conroy, 2012).

3 Results

Unless otherwise stated, the PFT and full-FT cohorts have been combined for this analysis. The distribution of cEQ scores with FT and control groups often deviated from normality based on Shapiro-Wilk statistic ($p < 0.05$ criteria) reflecting the skewed nature of the cEQ measures. While the delta cEQ scores passed this normality criteria, postflight scores did appear slightly bimodal (Figure 1) reflecting the variability in responses. Preflight performance was similar across groups. For SOT 5, preflight median cEQ score (±IQR) was 85.6 ± 6.5 for control and 90.6 ± 4.7 for FT ($p = 0.91$). For SOT 5M, preflight median was 78.6 ± 6.3 points for control and 81.8 ± 19.6 for FT participants ($p = 0.51$). Postflight two FT subjects completed only 2 of 3 SOT-5M trials; otherwise, all participants completed three trials for both SOT5 and SOT-5M in all sessions. Based on paired Wilcoxon signed-rank tests, FT and control groups had significant pre-to-postflight decrements in cEQ for both SOT5 ($p < 0.01$) and SOT5M ($p < 0.001$).

While these group decrements are consistent with previous ISS findings (Wood et al., 2015), from inspection of Figure 1 it is evident that some FT participants tended to perform closer to or even better than their preflight baselines. The median (±IQR) delta cEQ scores for SOT-5 for the controls were -8.5 ± 10.1 compared to -4.4 ± 7.0 for the FT group ($z = 0.99$, $p = 0.32$). The mean delta cEQ scores for SOT-5M for the controls were -39.0 ± 28.0 -compared to −24.5 ± 49.2 for the FT group ($z = 1.03$, $p = 0.30$). The probability that an observation from the FT group (with both full and pilot subgroups combined) was greater than the control group was PS = 0.61 for SOT-5 and PS = 0.62 for SOT-5M. Note that the two participants included in both FT and control groups performed better following FT participation. Improvements in post-flight performance were noted among both PFT and full-FT cohorts, although the greatest difference was between the pilot-FT and controls during SOT-5M ($z = 1.25$, $p = 0.21$, PS = 0.67). Nevertheless, the distribution of difference scores in the FT group, particularly for SOT-5M, appeared bimodal with some FT participants among the most impaired (Figure 1). The observation that some of the worst FT performances on SOT-5M were in the full-FT subgroup may reflect that the additional tasks required for the full protocol were more likely to exceed motion thresholds for some participants. Among other factors that may have contributed to postflight performance, we found that the number of flights ($r_s = -0.02$, $p = 0.92$), mission duration ($r_s = 0.01$, $p = 0.95$) and timing of the post-flight CDP ($r_s = 0.02$, $p = 0.93$) were all not correlated with the delta cEQ scores for SOT-5M across FT and control cohorts.

4 Discussion

Our post-flight CDP measures reflect the high intersubject variability that characterize postural decrements following spaceflight (Wood et al., 2015). This variability is consistent with other measures obtained during the Field Tests (Reschke et al., 2020) as well as previous studies (Miller et al., 2018; Mulavara et al., 2018). Nevertheless, multiple test sessions on landing day, starting early at the recovery zone, anecdotally appeared to be beneficial for some participants. Comparison of FT and no-FT participants in the most vestibularly challenging CDP condition support these anecdotal reports. We infer from these observations that performing minimal, challenging sensorimotor tasks very early in recovery provided enough challenge to the sensorimotor system to accelerate readaptation in some crewmembers.

This incidental discovery is not particularly surprising. Early ambulation is known to improve recovery outcomes following surgical interventions (e.g., Oldmeadow et al., 2006). While an early intervention is complicated by increased motion sensitivity at landing, similar interventions have proven useful clinically with motion sensitive vestibular patients (e.g., acute peripheral loss, Michel et al., 2020). This is also consistent with the observation that systematically increasing head movements during Shuttle reentry, as long as maintained within one's threshold for motion tolerance, anecdotally appeared to improve recovery (Wood et al., 2011). Performing head tilts too rapidly or with too much amplitude can exacerbate symptoms and illusory sensations (Small et al., 2012); conversely, restricting head movements can delay readaptation. The seemingly bimodal distribution of responses suggests that FT participation did not improve recovery in all subjects, illustrating the importance of maintaining activity within an individual's threshold. Our test protocol generally followed an incrementally challenging test sequence. Further improvements would be expected if the focus were on rehabilitation and customization of task difficulty.

There are limitations of this type of retrospective analysis. First, there is the possibility of self-selection bias for those consenting to participate in the Field Tests. Since ambulation was not quantified apart from the test sessions, it is unknown how much difference there was between groups. As noted in the methods, there was assisted ambulation for all participants who stood from sitting and lying positions, showered, and used stairs at the airports as part of their daily activities. Participating in the Field Tests likely had the greatest impact in early ambulation at the medical tents. Our comparison is made within an operational context with different medical interventions across subjects (Lee et al., 2020). The limited sample available as well as variations in CDP postflight test schedule also limit group comparisons.

The potential benefits from early mobility on landing day can be inferred from both anecdotal reports of the participants and comparison of the postural performance with no-FT participants. This finding has implications for exploration design reference mission planning. Instead of delaying planetary surface operations to allow for recovery, our results suggest that early mobility may be important. Rehabilitation should be optimized, as the tasks performed during Field Test were created to simulate aspects of mission-critical functional movements and were not intended as rehabilitation. Early active retraining, individualized based on the level of initial impairment, will enable a more efficient motor learning to the new environment (Lacour, 2006). Additionally, the rehabilitation should be phased appropriately, starting with simple tasks that grow in complexity with ability and time. A self-administered approach should provide optimized, graded tasks, e.g., beginning with low range-of-motion movements such as finger-to-object targeting practice and small postural changes, and advance to dynamic balance challenges. These should be performed as soon to landing as possible. It is also critical that the astronaut is coached to never exceed a motion sickness level around malaise, as once surpassed nausea and vomiting may not subside for hours. The importance of structuring rehabilitation exercises and early operational activities using *individualized* aversive threshold limits is underscored by our participants who may have impaired their recovery in an effort to complete all FT tasks, even when some tasks provoked motion sickness. These guidelines derived from our Field Test experience provide a framework to optimize performance for early mission success following G-state transitions during future space exploration. The bimodal response to FT participation has implications for vestibular rehabilitation on Earth; namely that early retraining must be individualized to promote adaptation while avoiding aversive conditioning.

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: NASA Life Science Data Archive and Lifetime Surveillance of Astronaut Health (lsda.jsc.nasa.gov)

Ethics statement

The studies involving human participants were reviewed and approved by NASA Institutional Review Board. The patients/participants provided their written informed consent to participate in this study.

Author contributions

MFR conceived of the study. MJR and SW conducted the data analysis. All authors contributed to the article and approved the submitted version.

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Conflict of interest

MJR was employed by the company KBR.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Applications of methods of psychological support developed for astronauts for use in medical settings

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Over the past 40 years, psychological support (PS) for cosmonauts and astronauts has remained an important part of the regular biomedical provision of space crews during extended orbital flights. It includes well-developed principles and a set of methods that have proven its effectiveness for the maintenance of behavioral health under extreme conditions of space flight. The main principle of PS in flight is to restore the usual sensory input to compensate for the monotony and lack of external stimuli as a result of a long stay under isolation and confinement. Risk factors for the psychological health and well-being defined for the astronauts, such as sensory and social deprivation, monotony, confinement, and lack of privacy, also remain part and parcel of several civil professions. These include polar wintering, submarines, working on oil platforms, and ocean fishing. Most of these factors also adversely affect the recovery rate of a large contingent of medical institutions, especially bedridden patients with chronic diseases. Finally, due to the negative epidemiological situation associated with the spread of COVID-19, an increasingly wide range of citizens forced to be in self-isolation faces negative manifestations of the deprivation phenomena described previously. Several cases of successful use of PS under isolation, monotony, crowding, and confinement are presented. Thus, we assume that the use of psychological support methods developed for space flights could be extremely relevant in civil medicine and everyday life.

KEYWORDS

psychological support, isolation, monotony, sensory deprivation, social deprivation, COVID-19, countermeasures, virtual reality

Introduction

The crews of long-term space expeditions live and perform complex operator activities under conditions of constant exposure to adverse factors of space flight, which pose a real threat to life and health. To counteract the adverse factors of space flight, Soviet specialists in the 70s of the 20th century developed a system of measures for psychological prevention and correction, conventionally named "psychological support". According to Kozerenko et al. (2001), psychological support (PS) for space crews is defined as a set of psychological methods, tools, and measures used by the mission control services to preserve the psychological health and mental well-being of astronauts under unfavorable conditions of extended space flight. The psychological support system was built in such a way that each group of unfavorable factors of space flight is opposed to a corresponding system of preventive measures (Gushin et al., 2020).

Being in orbit is associated with a prolonged stay in microgravity in a confined small chamber environment. This deprives the astronaut of the usual influx of visual, auditory, and tactile stimuli of the natural terrestrial environment, as well as a complex of vestibular signals associated with the effects of planetary gravity (Kanas, 2015). Along with the decrease in the volume of incoming information in flight, its diversity also decreases. It is caused not only by the monotonous environment created by life support systems but also by the flight protocol with frequent repetition of stereotypical actions for the maintenance of station systems (Kanas et al., 2009). Therefore, the appearance of psychological problems is associated with the processes of asthenization of the central nervous system, progressing decrease of its tone under sensory deprivation and causing inadequate reactions to incoming stimuli (Myasnikov and Stepanov, 2002). Its manifestations were vividly described for astronauts and polar winterers and could lead to increasing fatigue, deterioration of cognitive functions, stereotyping of actions, the appearance of anxiety, boredom, and loss of motivation and morale (Palinkas and Suedfeld, 2008). Psychological support measures for reconstruction of the usual sensory influx include prevention of the occurrence of sensory deprivation caused by the combined effect of sensory deprivation and monotony. They include regular provision of the crew members with the additional sources of external stimuli like fresh news, books, movies, music, and videos from Earth.

Another important group of unfavorable factors of long-term space flight is defined in the works of M.A. Novikov and his colleagues as the imposed nature of communication and the restriction of social contacts (Novikov, 1970). In flight, crew communications are an element of professional activity and are associated with the mandatory informing of the MCC about the life of the crew and receiving instructions and recommendations. An increase in the volume of mandatory professional communication is combined with a decrease in the number and duration of contacts with the cosmonaut's inner social circle. A person in orbit may lose part of the usual social support from relatives and friends. As a result, a feeling of loneliness and isolation from the usual environment appear, sometimes having an adverse effect on the family life of an astronaut. (Suedfeld, 2018). The conditions of crowding, combined with a high level of stress, can provoke conflict tension in a small group, a struggle for leadership in the crew. Therefore, another area of psychological support is the preservation of the usual circle of communication, maintaining the social ties of the crew in flight. It is maintained by the PS specialists who organize regular video conferences with families, friends, and VIPs for the astronauts (Johnson, 2010). Also, mission controllers are aware that crew members could transfer anger and aggression outside to prevent conflict within the crew (Kanas et al., 2009). They are ready for this draining of negativity and could even provoke it intentionally, to decrease psychological pressure within the crew.

Space and polar expeditions also lead to the fact that crews are forced to stay under conditions of crowding and lack of privacy. It should be noted that crowding and lack of privacy are problems relevant both for staying under lockdown conditions (due to a new coronavirus infection) and for a number of extreme professions (for example, employees of oil rigs in the open sea)—and for the modern urban environment as a whole.

At orbital stations, the problem of lack of privacy is solved by allocating personal spaces (cabins) for astronauts, but such solutions are limited by a shortage of resources and a small habitable volume of orbital stations.

Social isolation and restriction of freedom, as well as the phenomenon of crowding, contradict the socio-psychological nature of man. As a rule, people face similar conditions in exceptional situations. Closed space and limited group interaction can be observed while working on submarines, on Arctic and island expeditions, and in spacecraft and stations, as well as during stay in places of imprisonment and hospitalization. Research conducted back in the 1960s and 70s by I. Altman showed that for people who stay in small residential areas for a long time, the territoriality of behavior is characteristic, and violation of boundaries or rules of behavior in a group provokes conflict situations. Studying the behavior of people under conditions of crowding, I. Altman introduced the concept of privacy, which means "selective control of access to oneself" achieved by modifying the environment, which includes both physical and socio-cultural components. Privacy is ensured through various forms of verbal and nonverbal behavior. Later, in the course of an experiment simulating the work of astronauts under conditions of extreme crowding, the phenomenon of the transition of personal intersubjective territory into the category of interpersonal space was revealed, while the problems of living together in these conditions are solved by personalizing individual and group territories. Further studies conducted, in particular, on densely populated urbanized areas, confirmed the relevance of psychological problems associated with crowding, violation of psychological boundaries, and lack of privacy. The predominance of a negative psycho-emotional background (an increase in the level of aggression) develops as a result of the difficulty in realizing the normal, physiologically conditioned, human need for personal space (the need for territoriality), as well as a result of physical violation of personal boundaries, lack of space, and isolation. The results of the research study show that crowding and high social density can give rise to a feeling of anxiety in a person and worsen mood (Saegert, 1975). At the same time, men experience more negative emotions under conditions of increased density than in situations of reduced spatial density, whereas reduced density has a more depressing effect on women (Freedman et al., 1972). Studies show that prolonged stay of men in space with high crowding increases the level of cortisol in the blood (Heshka and Pylypuk, 1975).

Thus, in isolation, stable asthenization is formed under the influence of sensory deprivation, monotony, the forced nature of communication contacts, increased inactivity, and hypokinesia. It is quite natural that a person's emotional reaction to isolation and crowding is usually sharply negative.

Usually, several terrestrial models are used as analogs of space flight, like polar expeditions or chamber isolation studies (Palinkas et al., 2000). Vice versa, we describe several simulations and models that are commonly used in space medicine as analogs for the civil medicine cases and practices in the following sections. The objective of this study is to confirm the effects of space PS under conditions that simulate both space flight factors and medical conditions and the practice of civil (terrestrial) medicine.

Experimental models and research results

Model 1—dry immersion

Dry immersion (DI) is one of the most widely used ground models of microgravity. DI accurately and rapidly reproduces most of the physiological effects of short-term space flights. Within this model, healthy volunteers are placed, individually or in pairs, in a supine position in a covered bath that was filled with water at a temperature of 33 ± 0.5°C. The daily routine is specified in accordance with the schedule of studies and countermeasure procedures (if the experiment included them), including 8 h of sleep, 3–4 meals, a medical supervision program, and experimental studies. The model simulates such factors of space flight as mechanical and axial unloading as well as physical inactivity (Tomilovskaya et al., 2019). We regard dry immersion as a perfect simulation of staying of bedridden patients in the hospital (i.e., after surgery). In both cases, subjects are limited in motor activities, sensory and socially deprived, and have problems with organization of their free time.

The negative impact of hypokinesia on mental health both under the conditions of model experiments including DI and extended stay in the hospital and under the conditions of space flight arises as a result of a discrepancy between the afferent (sensitive) link of the central nervous system under load and another link (effector, i.e., motor), limited by the load of the general functional chain of motor acts. Negative, subjectively "difficult" mental states perceived by recipients may be expressed in neurotic manifestations. In particular, during the period of acute adaptation to the conditions of the experiment, irritability, complaints, increased conflict tension with the experiment staff, increased attention to one's own proprioceptive sensations (including pain), sleep disturbances at the stage of adaptation to dry immersion, boredom, and problems with organizing free time can be observed (Rozanov et al., 2022).

In October–December 2020, a 3-day dry immersion with the participation of six women from 24 to 39 years old (the average age is 30 years) was carried out on the basis of the IMBP RAS. The study was approved by the Commission on Biomedical Ethics of the IMBP RAS (Protocol No. 544 dated 06/16/2020). The recipients signed informed consent. It should be noted that the duration of immersion exposure (3 days) allowed us to consider this entire time interval as a period of acute adaptation to stressful experimental conditions. In the 3-day dry immersion aimed at studying the physiological and psychophysiological state of the women's body under the conditions of modeling the factors of space flight, the method of psychological support based on VR technologies was tested.

The objective of our study was to test the PS complex based on VR technologies and to study the psychophysiological effects that occurred in the recipients when they interacted with this complex. In order to meet the safety criterion, preliminary training of the subjects was conducted. According to the data of medical (neurological) examinations after VR sessions, no violations were detected. The psychological safety of the content was ensured through the preliminary censorship of audiovisual materials. The personification of the presented content was achieved through a preliminary questionnaire about preferences and the subsequent formation of a "repertoire" based on their analysis. The principle of matching the correspondence of the objective function was applied. Autonomous, i.e., not requiring connection to an external computer, VR helmets with properties such as lightness of construction, mobility, and ease of use were used. In addition, this "repertoire" was compiled taking into account the nature of the impact of the experimental conditions: immobilization, staying in an unsupported position, prolonged (almost constant) staying in a supine position, redistribution of body fluids, exceptionally small sensory influx through a number of sensory systems, and partial social deprivation (narrowing of the circle of social contacts and the predominance of forced and professional communication). Thus, to prevent active locomotion while perceiving visual images, presented pictures should simulate flying or gliding in an environment with low resistance, like atmosphere or water. As a VR environment, we used three-dimensional video, i.e., the content was non-interactive, not requiring active movements and other motor activity to interact with it—which is also necessary from the point of view of maintaining the purity of the experiment. Based on this, the content of the videos included flights above the planet's surface (both characteristic of the place of residence of the subjects and exotic) and swimming in the sea among marine animals (Rozanov et al., 2021; Rozanov et al., 2022).

We used non-invasive and objective research methods to assess the psychophysiological effects of PS. The participants recorded semi-structured self-reports about their health, mood, and working capacity. They were analyzed using Noldus FaceReader software and speech content analysis to get the data about their emotional status and cognitive functions (Кьntzler et al., 2021). With the help of this complex, the severity of the emotionality of the subjects was assessed in comparison with a calm facial expression. FaceReader software is able to evaluate the following indicators based on the facial expressions of testers: neutral (lack of emotions), valence and arousal, and emotions such as happy, sad, angry, surprised, scared, and disgusted. This hardware and software complex, as well as the corresponding computerized method of analysis, is an objective, validated method, widely implemented in the practice of psychological research. Actigraphy was applied to analyze subjects' locomotion and sleep duration by sleep analysis using the Cole–Kripke algorithm (Cole et al., 1992), calculations based on which were carried out using ActiLife software. Also, specially developed questionnaires were used to obtain the data about subjects' perception of the level of PS provided by the on-duty team (doctor, technician, and laboratory assistant) and the VR sessions. The questionnaire "Immersiveness" was developed in order to evaluate the subjective "presence effect" that occurs in testers during a virtual reality session (synonyms—immersion effect, immersiveness).

For statistical analysis of this model study, methods such as the calculation of the Wilcoxon and Spearman criteria were used, while the computer analysis was carried out in the SPSS program.

Results. In the self-reports during immersion, subjects, as expected, often complained about increasing fatigue caused by a "tightly" compiled protocol of studies, boredom, and sleep disorders. These types of complaints were mentioned in 23.3% of reports. Also, discomfort associated with difficulties in performing urination and defecation was mentioned. To conclude, staying in dry immersion in general negatively affected their psycho-emotional state.

According to the data of medical observation of the subjects after the VR sessions, four of them had a slightly pronounced pendulum-like fine-grained binocular mixed nystagmus of the first degree that can be regarded as physiological, not pathological, nystagmus. Two recipients did not report nystagmus at all. Nystagmus took place for 0.5–1.5 min after the end of the VR session. During the use of VR technical means and the selected visual content, no cases of nausea, dizziness, motion sickness, or coordination disorders were detected in the subjects who were under the conditions of DI.

To assess the impact of VR sessions on the cognitive and emotional spheres, detection of speech errors and filler words (or hesitation marks, which are so-called in linguistics as discursive and are markers of stress) in the transcripts of self-reports was carried out. It was found that the proportion of parasite words and speech errors in self-reports after the VR session decreased significantly (T = -2,201; $p < 0.05$). So, according to self-reports, a positive effect of VR sessions on the cognitive functions of the subjects was also found (Rozanov et al., 2022).

To estimate changes in the psycho-emotional status during dry immersion, a comparison of the average values of basic emotions was made using FaceReader. Facial expressions were recorded during DPC (daily planning conference, afternoon planning conference in the form of a structured self-report) of the recipients as well as before and after the VR session. A statistically significant tendency to increase the proportion of the neutral component in facial expressions was found (t = 3,155; p = 0,008). After the VR session, the proportion of neutral components in facial expression increased even more and was also statistically significant (T = 2,605; $p < 0.05$). At the same time, the indicator of the level of arousal statistically significantly decreased after the sessions (T = -0.973; $p < 0.05$). In other words, they were looking calmer and less tensed throughout the stressful conditions that can be an indicator of gradual adaptation to the experimental conditions. From that point, the impact of VR can be regarded as relaxing.

The effect of utilization of non-interactive VR content was also manifested in a decrease in the general volume of motor activity during the PS session. That was confirmed by the fact that the maximum of the motion vector during VR sessions was on average 2.4 times lower than the maximum per day. The maximum of the motion vector after the VR session was less in 13 out of 15 cases, 1.3 times on average (T = -2.48; $p < 0.05$). We suppose that a pronounced decrease in motor activity (and corresponding more pronounced tonic relaxation) during dry immersion also could be related to the facial expression of the subjects. For example, we detected an increase in the level of happiness emotion found by FaceReader before and after VR, which negatively correlated with the ratio of the daily maximum motor activity (VM) (c = -0.741; $p < 0.01$). There was also a stable positive correlation between the degree of immersion in visual virtual images and the difference in the severity of the arousal emotional index before and after the VR session (c = 0.598; $p < 0.05$). This correlation suggests that more psychologically immersive images of VR, in all likelihood, caused a more pronounced decrease in the "arousal" index in the subjects.

The obtained results allow us to draw a preliminary conclusion about the general relaxing and calming effect of PS via VR that reduces mental tension caused by adverse psychological factors of dry immersion.

Model 2—isolation experiment

The COVID-19 pandemic has become one of the most severe crises for public health and society in recent times. With many adverse consequences, such epidemics are always associated with adverse consequences for mental health (Palinkas et al.., 2021). The critical reduction of sensory information may be also explained by the reduction of social contacts, which decreases the chance of additional verification of social reality (Alle and Bernsten, 2021). It can make it difficult to distinguish between reality and imagination, which leads to distortions of perception, derealization, and hallucinations. There are reasons to believe that social isolation associated with confinement and monotony under quarantine can have important psychological consequences in terms of increased psychotic symptoms and cognitive problems (Fiorillo and Gorwood., 2020).

Such problems were noticed in 2020 in Italy during the second month of national isolation (D'Agostino et al.., 2020). Patients without a psychiatric history could have hallucinations and a somatic illusion that they were infected with the SARS-CoV-2. That corresponds with the old isolation study made in the middle of the 20th century by the McDonnell Douglas Corporation (Gushin, 2020). A depressing psychological state is also caused by uncertainty about the future, which is associated with a lack of data about the time of complete recovery from the disease on a global scale, as well as with a large number of uncontrolled news. That is why researchers express concern about the negative impact of self-isolation on both individuals who are forced to face prolonged isolation for epidemiological or medical reasons and patients with mental illness, whose clinical outcome may occur with the risk of exacerbation of symptoms and possible relapse (Chaturvedi, 2020).

A short-term isolation (chamber) experiment can be regarded as a short quarantine of the family group or small professional group that was typical for the COVID pandemic.

The ESKIS experiment with 14-day isolation and crowding of the mixed gender crew was carried out in the chamber of 50 cubic meters at the ground-based experimental complex (NEK) SSC RF–IMBP RAS. Six practically healthy volunteers, four men and two women (age 23–45 years), participated in the experiment. Five of them had no previous experience in isolation experiments (Gushin et al., 2020; Nosenkova, 2021). The daily routine was specified in accordance with the schedule of studies, including the execution of operator's performance tasks, three meals, 8 h of sleep, a medical supervision program, and experimental studies. The main psychological factors affecting the subjects during the experiment are typical for chamber confinement studies (as well as isolation caused by epidemiological reasons): sensory deprivation, monotony, crowding, lack of privacy, the limitation of social contacts, and the forced nature of communication (Feichtenger et al., 2012). It is important to note that subjects executed performance tasks, which corresponds with a remote regime of working that was established during the pandemic.

The PS complex included PS sessions based on VR technologies and sessions of "classical" psychological support, similar to those used in orbital flights. These included providing crew members with personalized multimedia content to view on personal tablets. This content was served by videos of a documentary nature and relaxation orientation with views of nature or space (for the creation of a "flight image" for the crew members) and also art therapy sessions, which included drawing on suggested topics and coloring books for adults. Sessions of each type of PS were conducted four times during isolation for each crew member in accordance with the cyclogram of the experiment.

For VR-based PS sessions, a special hardware and software complex was provided based on an autonomous portable VR helmet, and special software for this helmet was developed (and tested for the first time under extreme conditions in this experiment). This software was provided by IBMP specialists in cooperation with AI Health LCC. It allows users to transfer to a virtual analog of the personal space: with the help of VR tools, an interactive image of a personal recreation room is created. In this virtual room, the user can make changes to the interior, change the weather and time of day outside the window of the room, and control the fireplace according to personal preferences. A set of personal multimedia content is available from this room for listening and viewing.

We supposed that subjects, who for the first time faced a complex of factors of isolation in a hermetic object, lack of personal space, and crowding, may experience anxiety—a state associated with a sense of uncertainty (Gushin, 2020). According to space practice, PS under conditions of extreme confinement, crowding, and isolation can be obtained *via* contact with mission controllers (in this study, duty medical teams) or within the crew. Social support inside the cohesive crew is based, according to Weiss, on the "availability of people you can rely on" (Sarason et al., 1990). Another source of PS included the provision of personalized multimedia content to compensate sensory deprivation and monotony and organize leisure time. A VR complex was also used, identical to the one used in dry immersion.

We expected that subjects with better developed communicative skills and extroverted personalities would obtain PS mostly *via* contacts with the crew and supporting group. On the other hand, subjects with dominating introversion could have problems with getting social support to withstand sensory deprivation, monotony, and crowding. For them, PS *via* computerized content, including the virtual reality environment, which is clearly dosed, contains preliminarily defined relaxing data and provides an additional virtual space, should be the most preferred type of psychological support.

The research methods were similar to those in 3-day dry immersion. Also, Keirsey Temperament Sorter (KTS) was utilized to estimate the influence of personality traits on the preferences of PS type. The questionnaire "psychological support", which was also used in the 3-day DI, was supplemented for this experiment with new graphs that allow us to assess the degree of support from communication with the crew and a sense of association with it. This change was dictated by the fact that in the isolation experiments, contrary to dry immersion, participants have contacts not only with the representatives of the duty teams but also within the crew (Sholcova et al., 2021). To study the social interactions in the crew and the cohesion of the crew, we applied a method for diagnosing the value-oriented unity of the group.

For statistical analysis in these model studies, we used the same methods as in the dry immersion model experiment.

Results. Undercrowding conditions, the indicator "extraversion" obtained using KTS had a positive stable correlation with the indicator of support from communication (i.e., social support) with the duty crew ($p = 0.896$, $p < 0.05$), and the indicator "introversion" had a negative relationship with the same parameter ($p = -0.896$, $p < 0.05$)—according to the questionnaire "psychological support." Thus, it can be assumed that the extroverted crew members subjectively experienced a higher level of support from communication (which corresponds to such an event of "classical" psychological support as the organization of private communication channels) than from instrumental methods of psychological support.

By analyzing the data of the Keirsey test, it was found that persons with a tendency to introversion, who did not feel unity with the crew and the need to communicate with the duty crew, were more susceptible to psychological support using virtual reality technology. The indicator "extraversion" obtained using the Keirsey questionnaire had a positive stable correlation with the indicator of support from communication with the duty crew ($r = 0.896$, $p < 0.05$), and the indicator "introversion" had a negative relationship with the same parameter ($r = -0.896$, $p < 0.05$). It should also be noted that the average values of communication positively correlated with both the degree of association of the subjects with the crew (perception of themselves as part of the group) and the level of support ($r = 0.888$, $p < 0.05$ and $r = 0.858$, $p < 0.05$, respectively). In addition, it was shown that the indicator of the need for generalization and interpretation of information correlated negatively with support from BP ($r = - 0.858$, $p < 0.05$). In addition to the indicator of introversion, the indicator of the need for specific information (sensation index according to Keirsey) positively correlated with the need for PS based on VR ($r = 0.888$, $p < 0.05$). Thus, it can be assumed that introverted crew members had a higher expressed need for such an instrumental form of psychological support as VR. The facial expression analysis also showed a reduction of the emotion of anger after the session in all subjects ($T = -1{,}78$, $p = 0{,}89$)).

A positive two-way correlation was also established between photo support and sympathy for the duty crew ($r = 0.852$, $p < 0.05$) and between photo support and reading support ($r = 0.865$, $p < 0.05$). We assume that under the conditions of narrowing the circle of social contacts inherent in isolation, such types of content as photos and books performed a socially substitutive role in the surveyed.

Actigraphy data indicate a deterioration in the quality of sleep in isolation. In all the subjects, except one, the duration of sleep steadily decreased during the experiment. All subjects, except one, had a tendency to decrease the time spent in bed during the night hours allotted for sleep, while all subjects, except two, had an increase in the number of night awakenings. According to actigraphy data, the use of psychological support using VR technologies could cause a decrease in the number of nocturnal awakenings (by 1.3–1.4 times compared to the average value for the experiment) and an increase in sleep duration (by 10–13.3% relative to the average value for the experiment).

Limitations of the study

As we have already pointed out, these models are quite correlated with a wide range of medical problems faced by physicians and clinical psychologists on Earth. But significant limitations of this study are associated with the small sample size and short duration of model experiments. However, 1) this situation is typical for model experiments that are difficult to organize, and 2) our research is one of the first objective studies of the psychophysiological effects of psychological support. Previously, the effectiveness of PS was confirmed solely on the basis of subjective self-reports of astronauts and the opinions of psychoneurologists supervising them during the flight. Nevertheless, work in this direction continues both in a number of new model experiments and in control groups. Also, the available objective scientific data presented in this article confirm the effectiveness of promising types of PS relevant for implementation in healthcare. The description of the planned scope of the introduction of PS methods is based on a systematic analysis of a wide range of scientific literature.

Expected scope of application of psychological support methods

It seems promising and expedient to apply psychological support methods for orbital space flights in a number of extreme professions, whose representatives are characterized by a long exposure (exposure) of risk factors similar to those during space flight. It should be noted here that these measures can be applied as part of a whole set of PS for representatives of extreme professions who have been under extreme conditions for a long time, in isolation, combined with a number of other factors, for example, crowding and monotony, or separately, during off-duty hours, to correct the negative impact of stress factors on the body and mind, for psychoprophylaxis and psychohygiena.

1) Conditions of social isolation, sensory deprivation, and monotony are a typical set of unfavorable psychophysiological factors accompanying the long-term stay of patients in hospitals. Having been immobilized for a long time, deprived of contact with nature and mundane environment stimuli, and with a narrowed circle of social contacts, such patients are increasingly plunged into depression and do not show active volitional efforts for a speedy correction. Under these conditions, the familiar visual and auditory environment reconstructed with the help of computerized content, including virtual reality technologies (pictures of nature, native homes, and art objects–museums and art galleries) can create an additional information influx compensating for sensory deprivation. Thus, the detrained nervous system can restore its activity. This will have a positive effect not only on the mood but also on the well-being of bedridden patients and stimulate the desire to return to a full and normal life, i.e., to recover.
2) Social security and patronage of the elderly and sedentary disabled are currently virtually devoid of any means of psychological support. A system of psychological support based on elaborate space psychology tools and methods can be used with the participation of social security personnel. It can expand the range of services provided to the elderly and disabled and improve their life quality.
3) Conditions of prolonged quarantine caused by the epidemiological situation, with prolonged stay in monotonous confinement and isolation conditions with social and sensory deprivation, negatively affect the tone of the central nervous system (Li et al., 2022). Similarly to the previous set of conditions, providing quarantined people with means of PS will favorably affect the functioning of the central nervous system and compensate unfavorable effects of extended sensory and social deprivation.

4) Autonomous performance under extreme living conditions (polar wintering and remote drilling, etc.) is in many ways similar to the conditions of long-term space flight in terms of a set of factors adversely affecting humans. Workers are affected by the same sensory and social deprivation and monotony (Suedfeld, 2012). As a result, in particular, there is an increase in depression, sleep disorders, and conflict tension in winter quarters, which adversely affects not only the state of health but also labor productivity.
5) Personnel of fishing vessels engaged in fish processing, which is characterized by sensory "hunger," being in crowded conditions and extremely pronounced monotony of activity (monotony).
6) Divers undergoing prolonged decompression in a pressure chamber, which is characterized by sensory hunger, monotony, the inability to organize active forms of recreation, difficulties with structuring leisure, and a decrease in intellectual (cognitive) abilities against the background of natural physiological processes inherent in decompression.

It should be noted here that the described measures can be applied partially or as a whole complex of PS to correct the negative effects of stress factors on the body and psyche, as an effective set of countermeasures.

The criteria for the application (applicability) of PS methods should include:

1) health safety;
2) psychological safety—the content and the stimuli provided should not contain audiovisual stimuli that cause fear, anxiety, and other negative emotions and mental states in recipients;
3) personification—the content and the stimuli provided must be individually formed taking into account the personal cultural needs of the recipient, as well as his current psycho-emotional state;
4) compliance with the objective function—prevention of the adverse effects of psychological factors of the altered environment (i.e., space flight, ground-based model experiment, autonomous activity in isolation, and quarantine), in which the use of the PS complex is planned.

In the case of developing a set of PS measures for representatives of a certain type of extreme professions, psychological risk factors inherent in a particular profession should be taken into account. In accordance with the principle of compliance with the target function, the developed set of PS measures should include specific methods aimed against specific risk factors, taking into account their specific weight.

In addition, it is necessary to take into account the expected exposure time (exposure time) of a factor and the expected duration of stay under altered, extreme living conditions. This may be, for example, due to the fact that the amount of multimedia content for "classical" psychological support should be determined in such a way that it is enough for persons undergoing psychological support for the duration of the entire isolation without the development of such effect—when users are bored by the PS method or content provided via PS session. For example, VR in dry immersion, according to the data presented previously, served as an effective countermeasure against sensory hunger. However, it is obvious that the provided set of relaxation videos will not be enough for long-term isolation, since the video repertoire was prepared with the expectation of an experiment duration of 3 days. At the same time, the available literature data on the group dynamics of psychophysiological indicators of persons in isolation should also be followed. These data can serve as a kind of predictive model. For example, a period of 3–14 days can be characterized as a period of acute adaptation to extreme, unusual, altered living conditions. Also, thanks to the experience of isolation experiments, the so-called phenomenon of the third quarter has become well known—increasing asthenization, conflicts within the crew, and a decrease in motivation to perform a flight task, combined with a loss of interest in interacting with external subscribers, which falls precisely on the third quarter of isolation (accordingly, having this information when planning a set of PS measures, for example, for a remote expedition, it is possible to work out a proactive set of measures timed precisely to the third of the expedition period).

It is necessary to take into account not only the totality and peculiarity of risk factors and the gender and cultural characteristics of the representatives of the small group for which the PS complex is being developed but also technical features, primarily related to the habitable volume of the small group's place of residence and the bandwidth of communication channels. It is obvious that an acute shortage of habitable volume will make it impossible to apply recommendations on active forms of leisure and sports for psychological recreation (this was modeled in the ESKIS experiment, and active games in a virtual reality environment served as a countermeasure to such an acute shortage of motor activity). If there are significant communication delays, irregular communication sessions, and low bandwidth of information exchange channels, it is necessary to take into account the complex of psychological support methods being developed for interplanetary flights and adopt from it a number of methods applicable under conditions of increased autonomy.

Conclusion

During two simulated studies, a new hardware and software complex developed for providing PS sessions underwent medical testing within the framework of the activities of the Pavlov Center for Integrative Physiology for Medicine, High-tech Healthcare, and Stress Tolerance Technologies. Observed psychophysiological changes in the psychophysiological status of subjects under stressful conditions allow us to state that PS measures, elaborated for the needs of space medicine, effectively provide psychological support for the people experiencing prolonged stress, caused by confinement, sensory deprivation, monotony, and social isolation. Obtained results give us the opportunity to recommend applying psychological support methods, elaborated for orbital space flights, for the needs of terrestrial medicine.

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 Commission on Biomedical Ethics of the Institution of the Russian Academy of Sciences, the State Scientific Center of the Russian Federation–Institute of Biomedical Problems of the Russian Academy of Sciences (SSC RF-IMBP RAS)–physiological section, and the Russian Bioethics Committee under the Russian Federation Commission for UNESCO. The patients/participants provided their written informed consent to participate in this study.

Author contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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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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Canadian aging and inactivity study: Spaceflight-inspired exercises during head-down tilt bedrest blunted reductions in muscle-pump but not cardiac baroreflex in older persons

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As part of the first Canadian aging and inactivity study (CAIS) we assessed the efficacy of space-based exercise countermeasures for maintenance of cardiac and muscle-pump baroreflex in older persons during bedrest. An initiative of the Canadian Space Agency, Canadian Institutes of Health Research and the Canadian Frailty Network, CAIS involved 14 days of 6-degree head-down tilt bedrest (HDBR) with (Exercise) or without (Control) combined upper and lower body strength, aerobic, and high-intensity interval training exercise countermeasures. Twenty healthy men and women aged 55 to 65, randomly divided into control and exercise groups (male control (MC, $n = 5$), male exercise (ME, $n = 5$), female control (FC, $n = 6$), female exercise (FE, $n = 4$)) (age: 58.7 ± 0.5 years, height: 1.67 ± 0.02 m, body mass: 70.2 ± 3.2 kg; mean ± SEM), completed the study. Cardiac and muscle-pump baroreflex activity were assessed with supine-to-stand tests. Wavelet transform coherence was used to characterise cardiac and muscle-pump baroreflex fraction time active (FTA) and gain values, and convergent cross-mapping was used to investigate causal directionality between blood pressure (BP) and heart rate, as well as BP and lower leg muscle electromyography (EMG). Seven of the twenty participants were unable to stand for 6 minutes after HDBR, with six of those being female. Our findings showed that 2 weeks of bedrest impaired skeletal muscle's ability to return blood to the venous circulation differently across various sexes and intervention groups. Comparing values after bed rest with before bed rest values, there was a significant increase in heart rates (Δ of +25%; +17% in MC to +33% in FC; $p < 0.0001$), beat-to-beat EMG decreased (Δ of −43%; −25% in ME to −58% in MC; $p < 0.02$), while BP change was dependent on sex and intervention groups. Unlike their male counterparts, in terms of muscle-pump baroreflex, female participants had considerably decreased FTA after HDBR ($p < 0.01$). All groups except female control demonstrated parallel decreases in cardiac active gain and causality, while the FC demonstrated an increase in cardiac causality despite a similar decline in cardiac active gain. Results showed that the proposed exercises may alleviate muscle-pump baroreflex declines but could not influence the cardiac baroreflex decline from 14 days of inactivity in older adults.

KEYWORDS

muscle-pump baroreflex, exercise countermeasure, postural hypotension, aging, cardiac baroreflex

Introduction

The elderly population is increasing globally (Vandewoude et al., 2012), and it is predicted that the number of individuals over the age of 60 will surpass two billion by 2050 (Wang et al., 2016). Aging is often characterized by a decrease in physical activity and an increase in sedentary behavior. Both of these changes in lifestyle aggravate the lack of functionality in physiological systems (Mechling and Netz, 2009), which can result in deterioration of overall functional health, such as through orthostatic hypotension (Chodzko-Zajko et al., 2009; Lanier et al., 2011). Concerns about increased rates of sedentary lifestyles in older people heightened with the imposition of limits to personal movements during the Covid-19 pandemic to reduce viral transmission. Research has also shown adaptations of physiological systems during spaceflight to be similar to aging (Convertino et al., 1989; Convertino et al., 1990; LeBlanc et al., 2007; Platts et al., 2009; Spector et al., 2009).

Spaceflight-induced weightlessness is known to reduce muscle size and strength and cause functional changes of the heart and blood vessels which alters circulating blood and interstitial fluid volumes, arterial blood diastolic pressure, ventricular stroke volume, left ventricular mass and, resetting of the carotid baroreceptors (Antonutto and Di Prampero, 2003; Goswami et al., 2013). These multi-system changes can negatively affect the astronaut's ability to perform mission-related tasks and increase the risk of loss of consciousness and fainting upon re-introduction to gravity (e.g., landings on the moon or Mars). Head-down tilt bedrest (HDBR), similar to space flight, removes the gravitational hydrostatic pressure created by standing and the stresses of standing and walking from the musculature, and can simultaneously decondition the cardiovascular and skeletomuscular systems. Thus, HDBR is a validated technique for simulating microgravity exposure, which enables us to track the changes in the relationship between these systems, especially the relationship of BP to muscular activation.

Since the early days of human spaceflight, physical exercise has been highlighted as a potential countermeasure to cope with weightlessness-induced deconditioning. The process of space adaption appears to be similar to those found with prolonged inactivity (Moore et al., 2010). Knowledge from the implementation of space-based countermeasures can provide important insight for those interested in medicine and rehabilitation. During space missions, an effective, multi-purpose, and non-invasive countermeasure for preserving muscles and cardiovascular components is essential. A detailed examination of these and their history in spaceflight and bedrest are presented in depth by Hedge et al. (Hedge et al., 2022).

On board the international space station astronauts use three different types/modalities of exercise equipment; cycle ergometer, treadmill, and advanced resistive exercise device (ARED) (Laws et al., 2020). Each exercise has a distinct purpose. Astronauts have relied on cycle ergometer exercising since the early days of spaceflight for a variety of reasons, including its ability to accurately measure work output by systematically altering pedaling resistance and for its benefits to the cardiovascular system (Convertino, 1996; Laws et al., 2020). Walking or jogging on the treadmill is also the most crucial factor in maintaining bone and muscle health as it can generate impact forces on body (Swain et al., 2021). Finally, the ARED offers a multi-purpose whole-body workout that includes back squat, sumo squat, sumo deadlift, shrugs, shoulder press, bench press, bicep curl, triceps extension, and single-arm row (Laws et al., 2020).

Understanding spaceflight-induced changes in the body (e.g., cardiovascular deconditioning and loss of skeletal muscle mass) are not just important for improving astronaut health; they could also contribute to the development of countermeasures and therapies that help people suffering from age-related conditions and diseases on Earth. Although space countermeasures are not necessarily appropriate for the elderly on Earth, as they are designed for relatively healthy and fit individuals, they can help geriatricians and rehabilitation specialists gain a better understanding of musculoskeletal and cardiovascular alterations and to establish a treatment/prevention program (Goswami et al., 2012; Goswami et al., 2013). Furthermore, age-related physiological changes are linked to hormones, exercise levels, diet, and illness, making it difficult to pinpoint the root causes of muscle loss and cardiovascular changes (Clément, 2011). Exploring the relationship between spaceflight countermeasure use and aging would thus shed insight on the aging process and give unique viewpoints and innovative techniques for incorporating into Earth medicine and rehabilitation. (Goswami et al., 2012; Blaber et al., 2013; Goswami et al., 2013).

Bedrest, which is best characterized by immobilization and confinement, has acted as an informative analogue to investigate the impact of inactivity on musculoskeletal and cardiovascular systems. It has been shown that bedrest in healthy older individuals can result in a reduction of muscle size and strength, as well as changes in the function of the heart and blood vessels. Previous literature has shown that only 10 days of bedrest in older persons induced remarkable muscle weakening including a loss in whole-body lean mass (−1.50 kg; p = 0.004), lower extremity lean mass (−0.95 kg; p = 0.003), and strength (−19 Nm s^{-1}; Δ of −15.6%; p = 0.001) which is significantly greater than seen annually in the average aging population (Kortebein et al., 2007). Furthermore, six-degree head-down bedrest (HDBR) has been shown to be an effective analogue of microgravity/spaceflight conditions to simulate cardiovascular and musculoskeletal systems' deconditioning (Goswami et al., 2015; Goswami, 2017). Due to limited resources for human spaceflight research, prolonged HDBR serves as an ideal experimental environment to study post-flight deconditioning in astronauts.

In this research, we investigated the impact of combined upper and lower body strength, aerobic, and high-intensity interval training (HIIT) exercise countermeasures designed for older persons (Hedge et al., 2022) on maintaining the cardiac and muscle-pump baroreflexes in healthy 55–65 year old men and women during 14 days of 6-degree head-down tilt bedrest (HDBR). This research was conducted as part of the Canadian aging and inactivity study (CAIS), supported by the Canadian Institutes of Health Research (CIHR), Canadian Frailty Network (CFN), and the Canadian Space Agency (CSA). In this paper we explore the relationship between biological sex and exercise intervention (four separate cohorts including males and females in both control and exercise groups) on the physiological interplay between the cardiovascular and musculoskeletal systems for blood pressure (BP) regulation. Previous research showed that both systems were severely impacted by bedrest following 60 days of HDBR without exercise in middle-aged males (Xu et al., 2020).

Our team has developed a series of techniques to study the significance of lower limb muscle activity in maintaining BP. For this purpose, we adapted the wavelet transform coherence (WTC) analysis (Garg et al., 2013; Garg et al., 2014; Xu et al., 2017) and convergent cross mapping (CCM) causality (Verma et al., 2017a; Verma et al., 2017b; Verma et al., 2018) methods to extract indices that characterize the interaction time (fraction time active, FTA), response gain value (gain), and control directionality (causality) among cardiovascular and postural measurements. We hypothesized that daily activation of the muscles associated with both posture and the muscle-pump would limit the decline in the muscle-pump blood pressure reflex in terms of coupling (causality), strength (gain), and activity (FTA). Similarly, it was expected that aerobic exercise would positively affect the cardiac baroreflex.

Materials and methods

Study design and testing protocols

This experiment was conducted at the Center for Innovative Medicine (CIM) of the McGill University Health Centre Research Institute (RI-MUHC). The study consisted of four 26-days bedrest campaigns (Figure 1), during which 5 to 6 participants per campaign were subjected to HDBR at −6° to simulate spaceflight-induced fluid shifts.

Half of the participants received an exercise countermeasure procedure during the HDBR, while the other half served as controls and received stretch and joint movement physiotherapy. Daily exercises consisted of a combination of three sessions of the following: HIIT, low-intensity aerobic activity, and lower-body strength exercises, resulting in forty-two exercises over the 2 weeks of HDBR with 60 min of daily physical activity (Table 1). A detailed description of the exercise protocols is provided by Hedge et al. (Hedge et al., 2022) along with the rationale for their implementation. Briefly, like in-space exercise programs, cycling and resistive training regimes along resistive bands were prescribed for preserving muscles and cardiovascular health (Hedge et al., 2022). In addition, HIIT exercise was incorporated into the bedrest exercise program (Hedge et al., 2022). Equipment was modified so that all workouts were conducted in a head-down tilt posture. The intensity of the exercise countermeasures was modified individually according to the participants' performance and tolerance, as measured by heart rate and BP throughout HDBR. Apart from the exercise sessions, there were no differences in standards of care between the two groups. Food intake was prescribed and monitored by the MUHC staff based on the nutritional requirements for the control and exercise groups as well as by biological sex (Supplementary Material). Participants were provided with liquids as part of their regulated diet and were allowed water hydration *ad libitum*.

Overall ethical approval for CAIS was obtained from the research ethics board of the MHUC. The study was registered as a clinical trial (NCT04964999: Microgravity Research Analogue (MRA): Understanding the Health Impact of Inactivity for the Benefit of Older Adults and Astronauts Initiative) in the US National clinical trial registry. Research and data collection associated with our component of the study was approved by the Office of Research Ethics at Simon Fraser University. The participants signed a written informed consent and agreed to be available at MHUC for the entire 26 days study period. The research was conducted in compliance with the guidelines and regulations of the above agencies and the declaration of Helsinki.

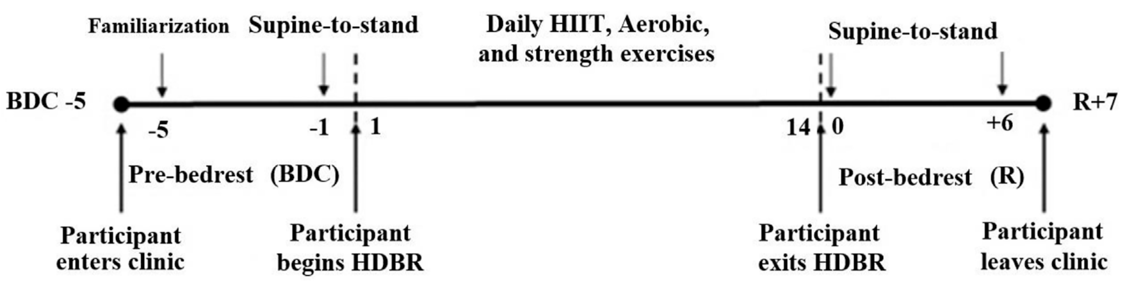

FIGURE 1
Timeline—the participants remained at the testing facility for a total of 26 days, of which 14 days were spent in 6° head-down tilt bedrest (HDBR). Participants arrived at MUHC 5 days prior to entering HDBR. At this time baseline data collection (BDC) was performed. After bedrest, participants remained at the clinic for 7 days where recovery (R) data were collected. A familiarization StS was performed on BDC-5 followed by research StS tests in the mornings of BDC-1 and R+0, R+6.

TABLE 1 Bedrest exercise protocols. A combination of up to three per day were performed with a maximum total time of 62 min per day.

Exercise	Type	Duration	Intensity	Total
Lower strength	Body weight, cables, resistance bands	25 min	12 times–max tolerance*	4
Upper strength	Body weight, cables, resistance bands	25 min	12 times–max tolerance*	5
Aerobic	HIIT	32 min (30 s on, 90 s off)	80–90% HRR	7
Aerobic	Progressive	15 min	30–60% HRR	14
Aerobic	Continuous	15 min	60–70% HRR	6
Aerobic	Continuous	30 min	60–70% HRR	6

Legend: *: By asking participants during each set; HRR: Heart rate reserve (max HR, resting HR, max HR, measured prior to bedrest).

Data collection

An electrocardiogram (ECG) was recorded with a bipolar three-lead ECG (IX-BIO4, iWorx, United States) in a standard Lead II electrode configuration. The non-invasive Portapres (FMS, Amsterdam, Netherlands) was used to monitor continuous BP at the finger, with absolute BP height-corrected to the heart level. Surface EMG was recorded transdermally from four bilateral lower leg muscles, including the tibialis anterior, lateral soleus, and medial and lateral gastrocnemius, using the Bagnoli-8 (Delsys Inc., MA, United States) EMG system. The SENIAM project's (Hermens et al., 1999) suggestions were used to select the locations for EMG sensor placement. Data were collected at 1,000 Hz using a National Instruments USB-6218 16-bit data capture equipment and LabVIEW 2013 software (National Instruments Inc., TX, United States).

Supine-to-stand test procedure

The phases of the study included 5 days of baseline data collection (BDC), 14 days of HDBR, and 7 days of recovery (R) (Figure 1). A supine-to-stand (StS) test was administered to activate and assess the cardio-postural control system (Blaber et al., 2009; Garg et al., 2013; Garg et al., 2014; Verma et al., 2017a; Rodriguez et al., 2017; Xu et al., 2017) twice during BDC and twice on recovery days. StS tests were performed in the mornings of BDC-5, BDC-1, R+0, R+6 (Figure 1). As the participants had not conducted an StS test in the screening process, the initial test on BDC-5 was considered a familiarization protocol for the participant. It should be noted that StS tests performed on BDC-1 and R+0 and were conducted 1 hour after the Canadian Space Agency (CSA) standard tilt test, which had a maximum duration of 15 min.

A room with no windows in a silent location was selected for the StS test to ensure participants' deprivation of auditory and visual stimuli during the protocol. Upon arrival at the testing room, the participants were placed in a supine position and instrumented for physiological monitoring. After instrumentation, lights were turned off and the participants were instructed to close their eyes while continuous data acquisition took place for 5 min. Following this, participants were asked to open their eyes and were assisted to the standing position. One researcher would sweep their legs off the bed, and another would assist with raising their torso. Participants' feet were placed parallel and 5 cm apart during standing. During the subsequent 6-min of quiet stance, they were instructed to keep their eyes closed with their arms relaxed at their sides, maintain an imaginary eye-level gaze, and not alter foot placement (Redfern et al., 2007).

Data analysis

In this study, we report the results from the stand portion of the StS test. The minute of data related to going from supine to stand was not utilized because of the existence of movement disturbance during the transition phase. At the end of this minute when the participant had their feet in the proper position, they were facing directly forward, and were standing free of assistance, the stand clock was started. We analysed the first 180 s of the stand to examine the reflex responses immediately following the transition period. The data analysis process has been previously elaborated in detail by Xu et al. (Xu et al., 2017). In summary, The ECG signal was used to calculate RR intervals. The maximum and minimum values in the BP waveform during a heartbeat were used to determine beat-to-beat SBP and diastolic blood pressure (DBP). Mean arterial pressure (MAP) was computed as the average BP from end-diastole to end-diastole of the waveform. Individual muscle beat-to-beat EMG (EMG impulse) was determined as the mean area under the rectified EMG envelope between successive heartbeats. The rectified EMG recorded from four separate muscles in each leg was summed to depict total muscle activity in the form of aggregate EMG. Before wavelet transform coherence and causality analyses, beat-to-beat physiological signals were interpolated using the spline approach and resampled to 10 Hz.

A Morlet wavelet was used to produce time–frequency distributions for the signal pair SBP → EMGimp (muscle-pump baroreflex) and SBP → RR (cardiac baroreflex) (Garg et al., 2013; Garg et al., 2014). Monte-Carlo simulation was used to determine the significant coherence threshold (Xu et al., 2017). In this research, the muscle-pump baroreflex was investigated in a low-frequency band (LF, 0.07–0.15 Hz) previously linked to cardio-postural coupling and the muscle-pump baroreflex (Xu et al., 2017). The vagal cardiac baroreflex (Blaber et al., 2022) was investigated in the high-frequency band (HF, 0.15–0.5 Hz). The area above the significant coherence threshold in each frequency band was divided by the overall area of that frequency band to calculate the portion of the total time with active interaction (Fraction Time Active: FTA). The cross wavelet transform of the two signals was used to obtain the response gain value (Grinsted et al., 2004) and averaged over sections of significant WTC within each frequency range. The effectiveness of each interaction was further described using "Active Gain", (Gain × FTA) (Xu et al., 2020).

The convergent cross-mapping technique was used to calculate the causal relationship between the signal pairs (EMGimp and SBP) and (RR and SBP) (Sugihara et al., 2012). Details on the methods may be found in Verma et al. (Verma et al., 2017a) and Sugihara et al.'s supplementary material (Sugihara et al., 2012). A two-dimensional plot (Active Gain vs. Causality) was utilized to show the correlation between causality and activity as they relate to the muscle-pump baroreflex and HDBR.

Statistical analysis

The interquartile range approach for detecting outliers was adopted to ensure that all cardio-postural values and interrelationship factors of BP and muscle activity were meaningful throughout the preprocessing stage. If a value was 1.5 times the interquartile range, larger than the third quartile, or less than the first quartile, it was termed an outlier. The *winsorization* approach to treating outliers was independently applied to each of the four participant groups (Kwak and Kim, 2017).

Given the small numbers of participants in each group (n = 4–6) from males and females who were randomly assigned to two interventions (control and exercise), where not all response variables were normally distributed, we used a nonparametric ANOVA-type statistic (nparLD, F2-LD-F1 design) suggested by Brunner et al. (Brunner et al., 2002). The F2-LD-F1 design refers to an experimental design with two between-subjects factors (sex and intervention) and one within-subjects factor (test days). This design was employed to study the effect of sex, intervention, and test days as well as their interaction on the calculated response variables. To investigate the pairwise differences between BDC-1, R+0, and R+6 (time main effect), we applied multiple comparisons (LD-F1 design) with Bonferroni adjustment. Kruskal–Wallis test followed by Conover-Iman post-hoc test was used to study the differences between male controls, female controls, male exercise, and female exercise (treatment main effects) during BDC-1, R+0, and R+6. All statistical tests were performed using R (Team, 2011), and data are reported as significant ($p < 0.05$) or trends ($0.1 > p \geq 0.05$).

Results

Participants

Following the screening of volunteers with inclusion and exclusion criteria (Supplementary Material), twenty-three participants entered the study. These participants were randomized by the RI-MUHC staff into the four test groups and then into four campaign cohorts: one cohort of five and three cohorts of six individuals. One participant withdrew from the study during the head-down tilt portion, and two others developed medical conditions unrelated to bedrest,

TABLE 2 Bedrest exercise protocols. A combination of up to three per day were performed with a maximum total time of 62 min per day.

Sex	Intervention	Presyncope	Reason for termination	Total stand time (s)	Data analysis segment (s)
Female	Control	Yes	sudden ↓BP	269	180
		Yes	sweating, participant request	250	180
		Yes	sudden ↓BP	209	180
		No	------	360	180
	Exercise	Yes	dizziness, sudden ↓BP	83	X
		No	------	360	180
		No	------	360	180
		Yes	sudden ↓BP	151	140
		Yes	sudden ↓BP	145	140
		No	------	360	180
Male	Control	No	------	360	180
		No	------	360	180
		No	------	360	180
		No	------	360	180
		No	------	360	180
	Exercise	Yes	sudden ↓BP	321	180
		No	------	360	180
		No	------	360	180
		No	------	360	180
		No	------	360	180

during the recovery phase and were removed from the study before completion. Therefore, data from twenty healthy men and women between 55–65 years of age were analysed (age: 58.7 ± 0.5 years, height: 1.67 ± 0.02 m, body mass: 70.2 ± 3.2 kg; mean ± SEM). The final group sizes were as follows: male controls (n = 5), female controls (n = 6), male exercise (n = 5), and female exercise (n = 4). All participants spent a total of 26 days (5 days of adaptation to the facilities, followed by 14 days of traditional six degrees of downward inclination bedrest in which participants used a pillow, and 7 days of recovery) at the Research Institute of the McGill University Medical Centre (RI-MUHC).

Presyncope

Seven of the twenty participants were unable to complete the StS test on R+0 (Table 2). Six of the seven non-finishers were female. The male non-finisher was in the exercise group and was 39 s from completing the total 6-min stand. The female non-finishers were evenly split between the exercise and control groups; however, participants in the exercise group had the shortest times to presyncope of all non-finisher participants. These three participants all had less than the standardized analysis window of 180 s for WTC and causality analysis. One participant with 83 s was removed and the other two were analysed using a 140 s window (Table 2) reducing the analysis sample size for the female exercise group on R+0 to five.

Cardiovascular and electromyography responses

The cardiovascular and EMG measurements were influenced considerably by 14-days HDBR. Given the small sample size per group, differences were found in the baseline values. To examine post-bedrest responses, we first compared values in each group to their baselines (Table 3), then responses between groups were compared using changes in values from BDC-1; increases being positive and decreases being negative (Figures 2, 3).

During the quiet stand of the R+0 StS test, 2 h after the end of bedrest, a significant increase from the BDC-1 baseline in the average HR was observed in all study groups ($p < 0.0001$) (Table 3; Figure 2), with a significant reduction towards baseline values on R+6 in female ($p = 0.019$) but not the male participants (Figure 2).

The response of standing SBP, DBP, and MAP differed between the intervention and sex groups throughout test days. On R+0 the male control group had an increase in systolic blood pressure while the female control ($p = 0.056$) and male exercise ($p = 0.068$) groups trended in the opposite direction (Table 3; Figure 2). No change from baseline was observed with the female

TABLE 3 Mean (± standard error) standing cardio-postural values for different groups including male control group, male exercise group, female control group, and female exercise group on BDC -1 and R+0. Mean cardio-postural values were obtained from the stand phase of the supine-to-stand test. BDC -1: baseline data collection day −1; R+0: 2 h after the end of bedrest; R+6: 6 days after bedrest; HR: heart rate; SBP: systolic blood pressure; DBP: diastolic blood pressure; MAP: mean arterial pressure; EMG: electromyogram; EMGimp: Electromyogram beat-to-beat impulse.

Variable	Sex	Pre-bedrest (BDC -1)		Post bedrest (R+0)		Post bedrest (R+6)	
		Control	Exercise	Control	Exercise	Control	Exercise
HR (bpm)	Male	77.8 ± 2.5	71.5 ± 3.0	91.8 ± 2.6 *	91.1 ± 4.6 *	86.7 ± 2.8	83.0 ± 3.2 *
	Female	74.2 ± 1.3	83.3 ± 1.2	99.0 ± 3.5 *	103.8 ± 2.7 *	79.1 ± 1.0 †	88.5 ± 2.0
SBP (mmHg)	Male	126.7 ± 8.6	141.6 ± 5.3	153.6 ± 2.7 #	121.9 ± 7.0 *	151.5 ± 4.1	149.8 ± 8.1
	Female	117.9 ± 3.5	147.8 ± 3.5	97.6 ± 3.0 #‡	143.8 ± 3.2 ‡	119.33 ± 5.4	123.1 ± 5.3
DBP (mmHg)	Male	66.1 ± 4.1	65.3 ± 1.7	81.9 ± 2.3 ‡	66.1 ± 1.0 ‡	64.2 ± 1.7 †	67.0 ± 2.5
	Female	63.3 ± 1.8	76.5 ± 2.0	59.2 ± 1.9 ‡	86.2 ± 2.4 ‡#	55.4 ± 3.1	66.5 ± 3.4
MAP (mmHg)	Male	81.7 ± 5.2	83.7 ± 2.5	99.8 ± 2.4 #	80.1 ± 2.5	86.1 ± 2.3	84.8 ± 3.1
	Female	80.9 ± 2.0	96.96 ± 2.2	71.8 ± 1.7 *#‡	101.7 ± 2.8 ‡	74.9 ± 3.4	83.3 ± 3.9
EMG (μV)	Male	193.5 ± 27.6 #‡	73.0 ± 3.9 ‡	105.2 ± 2.6 *	65.6 ± 6.4	89.8 ± 3.7 #*	63.0 ± 3.0
	Female	86.7 ± 6.3 #	92.9 ± 3.9	74.2 ± 5.4	74.2 ± 5.3	53.8 ± 3.5 #	69.98 ± 3.4 *
EMGimp (μV·s)	Male	162.7 ± 27.8 ‡	65.0 ± 5.6 ‡	67.0 ± 2.6 *	48.8 ± 7.4	63.9 ± 4.0 *	47.5 ± 3.4 *
	Female	70.3 ± 4.8	66.6 ± 2.5	46.1 ± 4.1 *	42.6 ± 2.5 *	40.6 ± 2.1 *	48.7 ± 2.6

Legend: *: significantly different from BDC-1, #: significant difference between male and female participants in the same intervention group., ‡: on each day, the control and exercise intervention groups were significantly different for the same sex. Significance was set at $p < 0.05$.

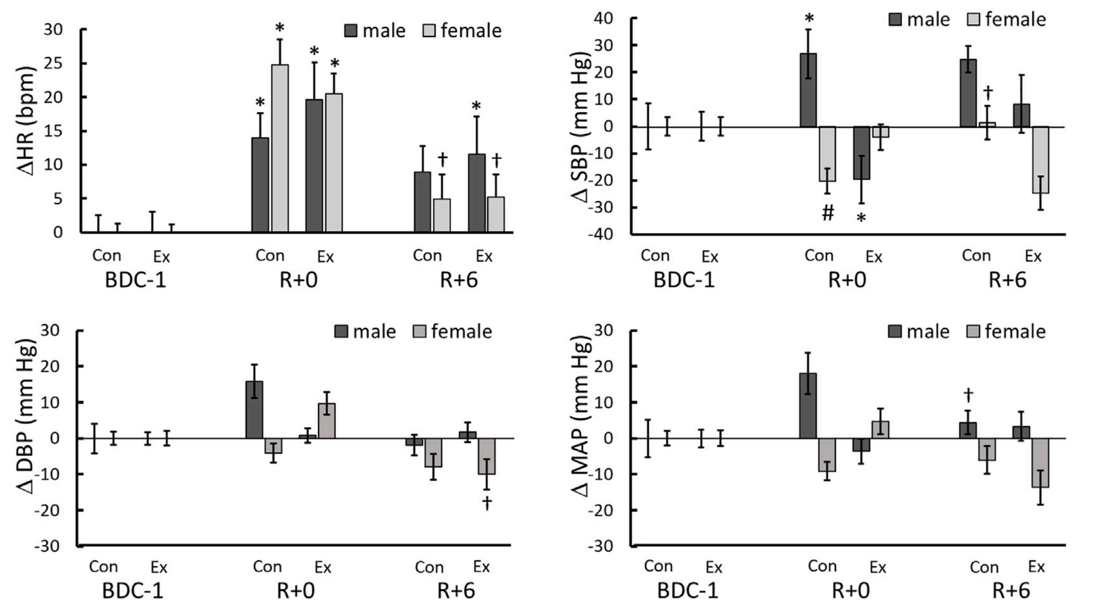

FIGURE 2
Heart rate and blood pressure changes from BDC-1 (increase: positive; decrease: negative) for different sex and intervention groups on R+0 and R+6. *: significantly different from BDC-1, †: R+6 different from R+0. #: different from males in same day and intervention. ‡: the control and exercise groups were significantly different for the same sex.

exercise group on R+0 or any group on R+6 (Table 3); however, significant reversals from R+0 occurred with the female control and male exercise SBP responses to standing. (Figure 2). No changes from baseline were found for DBP or MAP for all groups studied (Table 3); however, similar to SBP, there were trend reversals from R+0 to R+6 (Figure 2).

Overall lower leg muscle activity was only significantly reduced with HDBR in the male control group (Table 3).

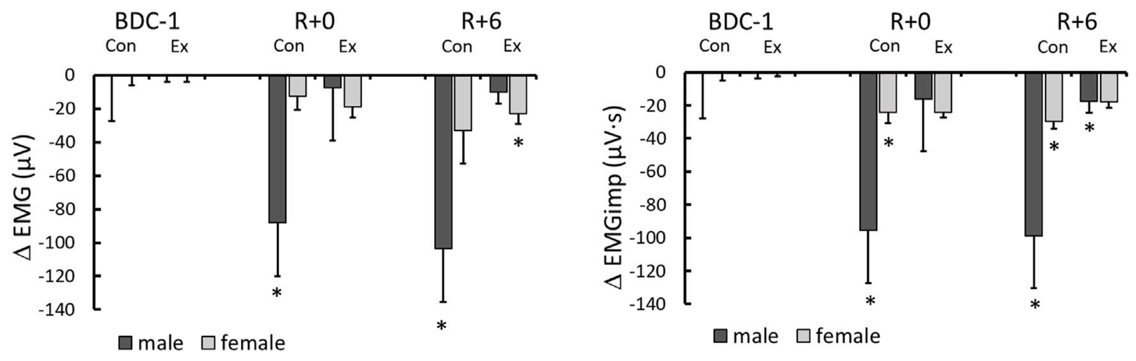

FIGURE 3
Electromyography (EMG) and electromygraphy impulse (EMGimp) changes from BDC-1 (increase: positive; decrease: negative) for different sex and intervention groups on R+0 and R+6. *: significantly different from BDC-1.

TABLE 4 Wavelet transform analysis and convergent cross-mapping of systolic blood pressure and calf muscle electromyography impulse interactions during standing for different groups including male control group, male exercise group, female control group, and female exercise group on BDC -1 and R+0. BDC -1: baseline data collection day –1; R+0: 2 h after the end of bedrest; R+6: 6 days after bedrest; Gain: wavelet transform gain; FTA: fraction time active (above significant coherence threshold); causality: control directionality; LF: low frequency. Values are means (± standard error).

Variable	Sex	Pre-bedrest (BDC -1)		Post bedrest (R+0)		Post bedrest (R+6)	
		Control	Exercise	Control	Exercise	Control	Exercise
FTA (LF)	Male	0.30 ± 0.05	0.37 ± 0.03	0.22 ± 0.02	0.13 ± 0.01 *	0.19 ± 0.07	0.21 ± 0.01 †
	Female	0.35 ± 0.07	0.21 ± 0.02	0.25 ± 0.08 *	0.12 ± 0.02	0.35 ± 0.10	0.12 ± 0.02
Gain (LF) (µV · s/mmHg)	Male	0.71 ± 0.07	0.95 ± 0.10	0.45 ± 0.04*	0.56 ± 0.03	0.68 ± 0.07	0.78 ± 0.07 †
	Female	0.71 ± 0.07	0.51 ± 0.03	0.76 ± 0.08	0.64 ± 0.08	0.62 ± 0.12	0.66 ± 0.12
Causality (SBP → EMGimp)	Male	0.85 ± 0.01	0.87 ± 0.01	0.73 ± 0.02*	0.81 ± 0.02	0.80 ± 0.02	0.77 ± 0.02*
	Female	0.87 ± 0.02	0.84 ± 0.02	0.80 ± 0.03	0.81 ± 0.03	0.87 ± 0.01	0.80 ± 0.02
Causality (EMGimp → SBP)	Male	0.90 ± 0.01	0.93 ± 0.01	0.91 ± 0.01	0.92 ± 0.01	0.88 ± 0.02	0.91 ± 0.01
	Female	0.93 ± 0.01	0.91 ± 0.01	0.90 ± 0.02	0.92 ± 0.01	0.9 ± 0.01	0.85 ± 0.01

Legend: *: significantly different from BDC-1, †: R+6 different from R+0.

However, when EMG was integrated beat-to-beat (EMGimp), the effect was more dramatic in both male and female control groups, with more than a 33 and 25% reduction, respectively, from baseline on R+0. These changes persisted on R+6 at similar magnitudes (Figure 3).

Muscle-pump baroreflex

Following HDBR, the skeletal muscle-pump's ability to react to variations in BP was significantly reduced (Table 4). The FTA response varied across intervention and sex groups throughout the test days. Male exercise and female control groups had a substantial reduction in FTA on R+0 compared to pre-bedrest values (BDC-1), while no changes from baseline were found for other groups. Only the male exercise group increased significantly on R+6 (Table 4). With respect to the muscle-pump baroreflex, where skeletal muscle responds to changes in BP, only the male control group showed a significant reduction in SBP→EMG gain on R+0 from baseline. Although not significantly reduced on R+0, the male exercise group was significantly higher on R+6 than R+0 and not different from baseline (p = 0.006). No change over HDBR or recovery was observed in female participants.

Cardiac baroreflex

Our data from the coupling of blood pressure and heart rate (SBP→RR) showed that the cardiovascular baroreflex was affected by HDBR (Table 5). The fraction that the cardiac baroreflex was active (FTA) was significantly decreased in females only after

TABLE 5 Wavelet transform analysis and convergent cross-mapping of systolic blood pressure and cardiac arterial interactions during standing for different groups including male control group, male exercise group, female control group, and female exercise group on BDC -1 and R+0. BDC -1: baseline data collection day −1; R+0: 2 h after the end of bedrest; R+6: 6 days after bedrest; SBP→RR: Neural cardiac baroreflex direction; RR→SBP: mechanical non-baroreflex direction; Gain: wavelet transform gain; FTA: fraction time active (above significant coherence threshold); causality: control directionality; HF: high frequency. Values are means (± standard error).

Variable	Sex	Pre-bedrest (BDC -1)		Post bedrest (R+0)		Post bedrest (R+6)	
		Control	Exercise	Control	Exercise	Control	Exercise
FTA (HF)	Male	0.46 ± 0.03	0.38 ± 0.04	0.35 ± 0.04	0.26 ± 0.02	0.39 ± 0.02	0.39 ± 0.01
	Female	0.36 ± 0.07	0.47 ± 0.06	0.22 ± 0.07 *	0.30 ± 0.06 *	0.42 ± 0.07	0.45 ± 0.06
Gain (HF) (ms/mmHg)	Male	5.09 ± 0.39	10.85 ± 1.25	2.56 ± 0.37 *	3.32 ± 0.30 *	3.03 ± 0.40 *	3.16 ± 0.17 *
	Female	9.43 ± 1.32	5.25 ± 1.10	3.13 ± 0.61 *	2.09 ± 0.30 *	5.63 ± 0.36	4.12 ± 0.64
Causality (SBP → RR)	Male	0.95 ± 0.01	0.95 ± 0.01	0.93 ± 0.01	0.91 ± 0.01	0.93 ± 0.01	0.92 ± 0.01
	Female	0.90 ± 0.017	0.88 ± 0.01	0.95 ± 0.01 *	0.88 ± 0.03	0.88 ± 0.03	0.89 ± 0.01
Causality (RR → SBP)	Male	0.92 ± 0.01	0.95 ± 0.01	0.93 ± 0.01	0.87 ± 0.03	0.93 ± 0.01	0.94 ± 0.01
	Female	0.94 ± 0.01	0.89 ± 0.01	0.91 ± 0.01	0.94 ± 0.01	0.91 ± 0.01	0.85 ± 0.01

Legend: * significantly different from BDC-1.

bedrest (R+0), but this recovered to baseline levels by R+6. The exercise intervention had no discernible effect on the outcomes as cardiac baroreflex gain was significantly reduced following bedrest on R+0 in all groups studied. The male exercise group had the greatest reduction in cardiac gain (~65% on R+0), and both male groups remained depressed on R+6, while both female groups had returned to baseline (Table 5).

Causality

Significant changes in SBP→EMGimp causality were only seen in the male study participants. On R+0, CCM analysis of SBP→EMGimp directional coupling (baroreflex) revealed a substantial reduction in causality in the male control group ($p < 0.0001$), which recovered by R+6. This reflex muscle-pump baroreflex causality trended lower in the male exercise group on R+0 ($p = 0.07$), but by R+6 this became significantly reduced from baseline (Table 4). In the opposite (muscle-pump mechanics) direction (EMGimp→SBP), there was no change in causality, with a value that remained constant at a mean value of 0.91 ± 0.01.

Causality for the female control group post HDBR cardiac baroreflex (SBP→RR) increased but returned to baseline by R+6. There was no change in male causality related to HDBR. There was also no change in the causal effect of heart rate on blood pressure (RR→SBP, cardiac mechanics) in any group.

Active gain vs. causality

To compare pre- and post-bedrest baroreflex responses, muscle-pump and cardiac baroreflex active gain, which is the product of gain and FTA (Gain × FTA), were plotted as a function of causality on BDC-1 and R+0 for all groups (Figure 4). There were different reactions in terms of baroreflex functionality between the intervention and sex groups pre- and post-bedrest. Regarding the muscle-pump baroreflex, when compared to BDC-1, the male exercise group had the greatest reduction in muscle-pump active gain, while the male control group had the largest decrease in causality (Figure 4A). Females in both the control and exercise groups had more mild results than males in the same group in terms of muscle-pump interactions (Figure 4A).

The intervention and sex groups' responses in cardiac baroreflex functionality were more dramatic following HDBR compared to muscle-pump baroreflex outcomes (Figure 4B). Although all groups studied had substantial decreases in cardiac baroreflex active gain, the only significant reduction was found in the female exercise group. The male exercise group, on the other hand, had both active gain and causality reductions following HDBR that were larger than the male control group. The female control group was the only group that exhibited a reversed direction of stronger causality and reduced active gain on R+0 compared to baseline on BDC-1 (Figure 4B).

Discussion

Our new findings from the Canadian aging and inactivity study highlight the detrimental effects of bedrest on homeostatic mechanisms responsible for functional daily ambulatory activities. This was particularly serious with the female participants of whom 60%, compared to 10% of male participants, were unable to complete 6 minutes of stand just hours after exiting from 14 days of bedrest. Given the drastic consequences on orthostatic tolerance, this paper is focused on

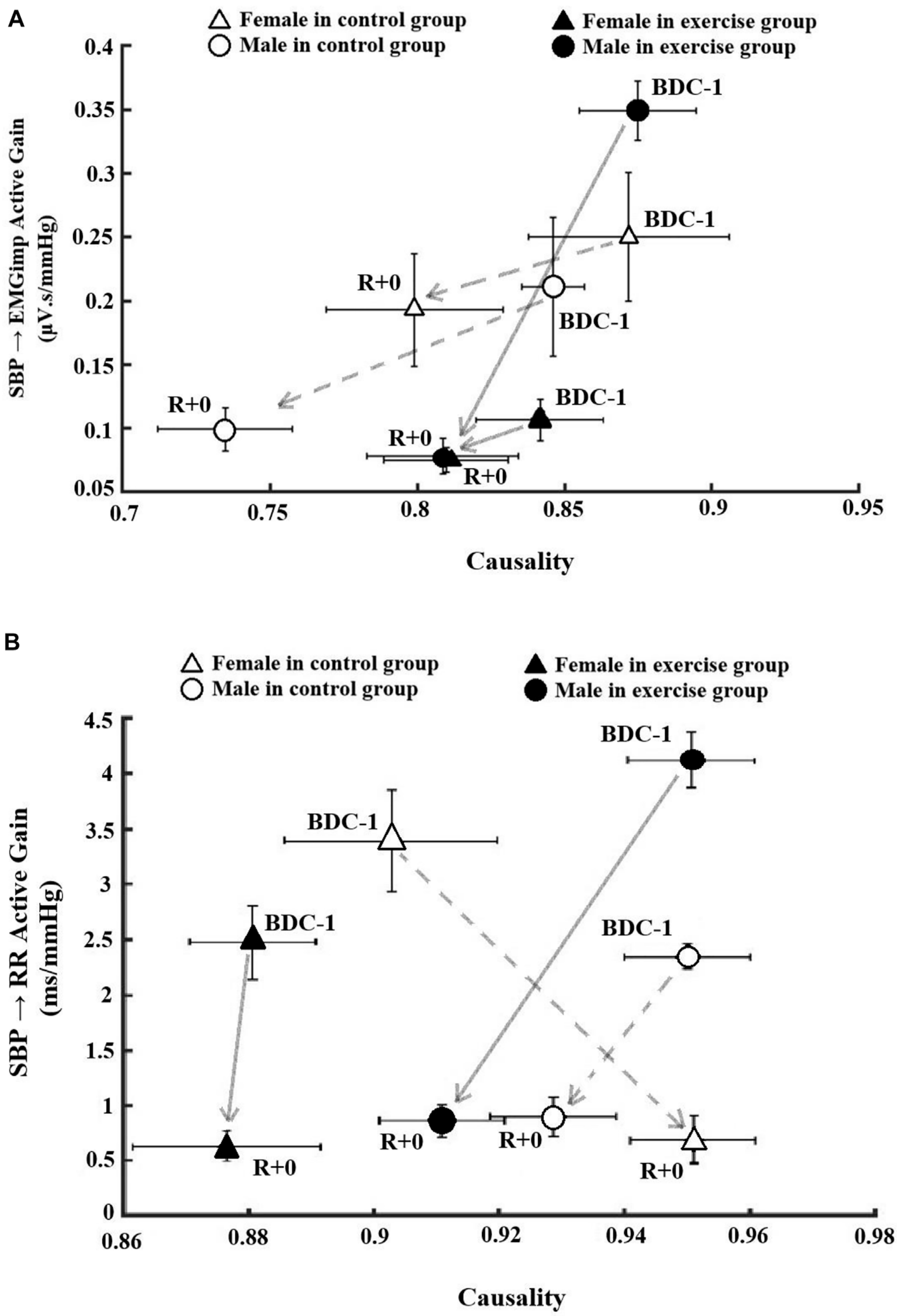

FIGURE 4
The association between causality and low frequency Active gain as a function of active interaction time (Active Gain: Gain X fraction time active) on pre bedrest (BDC-1) and R+0 related to **(A)** skeletal muscle-pump baroreflex system and **(B)** cardiac baroreflex system. The data in the circles are associated with male participants, while the data in the triangles are related to female participants. Filled markers indicate the exercise groups in both sexes.

two major components of the blood pressure control system, the cardiac and muscle-pump baroreflexes. Our results into orthostatic reflexes reveal that following 2 weeks of bedrest, skeletal muscle activation and heart rate changes in connection to BP regulation were reduced in older participants. To our knowledge, this is the first study to report changes in cardio-postural interactions in both sexes and older persons after extended bedrest confinement.

The findings are particularly relevant for understanding orthostatic intolerance (OI), a syndrome that affects both older (Goswami, 2017) and younger (Xu et al., 2020) people after bedrest, simulated microgravity (e.g. HDBR) (Goswami et al., 2017), or astronauts after spaceflight (Blaber et al., 2011; Blaber et al., 2013; Blaber et al., 2022), respectively. Furthermore, the older composition of participants in this study adds to our understanding of the interaction of age with inactivity on the cardio-postural control system.

Muscle-pump baroreflex

We recently provided evidence of the importance of lower limb muscular contractions for the maintenance of standing blood pressure (Xu et al., 2020). These contractions compress underlying veins, resulting in the pumping of venous blood pooled in the legs back to the heart (muscle-pump) in a coordinated response raising venous return to counteract reductions in BP (Verma et al., 2017a; Verma et al., 2019). As a result, BP management during standing necessitates input from the cardiovascular, postural, and musculoskeletal systems. We also showed that this blood pressure related muscle-pump reflex was impaired in middle-aged male participants following 60 days of bedrest inactivity (no exercise intervention) (Xu et al., 2020). This is the first study where muscle-pump baroreflex has been investigated in women following bedrest.

We used EMG impulse as an indicator of the beat-to-beat translation of muscle activity (EMG) to the cardiovascular system via the skeletal muscle-pump. Given the considerable variation in baseline EMG and EMGimp across groups (Table 1)—most likely related to the small sample sizes—changes from baseline were used to assess intergroup effects. EMGimp decreased significantly only in the male and female control groups on R+0 and R+6 compared to pre-HDBR values (Figure 3). Major declines in EMGimp were not observed in the exercise groups, although the male exercise group showed a significant decline on R+6, this value was not different from R+0, which had much higher variation, and not different from the female exercise group. Of the two sexes, males had the largest decreases in EMGimp. Males are predicted to have more muscular deconditioning and dramatic alterations since they have larger muscle mass than females. Smaller, yet consistent declines in EMGimp were also seen in the female control group. In contrast, no change in EMGimp from baseline was seen in male or female exercise participants (Figure 3), indicating that they were better able to sustain muscle-pump capacity after lengthy periods of bedrest. These data are a clear indication that the skeletal muscle's capacity to pump blood back to the venous circulation in response to BP variations was impaired by 14 days of bedrest but was preserved by the daily exercise regime.

Our previous research with healthy younger males found that along with reduced EMGimp there was a changed relationship of BP to muscular activation (gain, FTA, causality) after bedrest and inactivity which indicated not only a probable drop in reflex output to the muscle but also a variation in activation (Xu et al., 2020). In this study, we examined the variations among the four studied groups to see how biological sex and exercise intervention affected muscular activation through the muscle-pump baroreflex. Following HDBR, there was a considerable decrease in the percentage of significant coherence over the duration of the stand, as expressed through FTA, in the male exercise and female control groups. Given the small sample size per group and mixed results, these data implied that after an extended period of immobility, the prescribed exercises may have a lesser impact on preserving FTA in male participants, and improving the training parameters such as loading frequency, workload, rate, and rest period should be studied more closely.

Like EMGimp, muscle-pump (SBP → EMG) gain was reduced the greatest in the male control group; however, unlike EMGimp no reduction was observed in female controls (Table 4). In fact, there was an across-the-board retention of muscle-pump baroreflex gain in all female participants post-bedrest. The male exercise muscle-pump baroreflex gain on R+0 was not decreased significantly but showed a significant increase from R+0 on the last day of measurement (R+6) which may indicate a positive latent effect of exercise in the male participants (Table 4).

Causality, a measure of the strength of coupling between signals was reduced in the muscle-pump baroreflex direction (SBP → EMGimp) on R+0 and R+6 in the male control group only. This supports our previously reported reduction in coupling between blood pressure and the skeletal muscle-pump following bedrest in male participants (Xu et al., 2020). This decline in muscle-pump directional influence was not observed in the male exercise group or in the female participants. These data further solidify the beneficial effects of exercise in older males. No change in muscle-pump baroreflex causality in the female participants was observed, suggesting a possible sex-related differential effect of bedrest which was also observed in muscle-pump baroreflex gain. However, caution must be taken in interpretation given the small sample size and moderately short time in bedrest.

The absence of causality changes in the inverse direction (EMGimp → SBP) implies that HDBR did not affect the mechanical connection between muscle-pump activity and BP. These data add further support to our hypothesis that variations in BP control are reflex/neurally mediated rather than caused by changes in muscle-pump mechanics (Xu et al., 2020). Finally, we examined the interaction between muscle-pump activity, the product of gain and FTA, with causality. Figure 4A showed that regardless of baseline active gain, exercise limited the reduction in EMG to BP coupling, as shown by the greater changes in causality in the control groups.

Cardiac baroreflex

Another critical component of the autonomic response to sudden reductions in blood pressure upon standing, is the cardiac baroreflex. Efferent neural pathways increase heart rate, systemic vascular resistance, and cardiac contractility via vagal withdrawal and sympathetic activation. Reductions in cardiac arterial baroreflex response have long been recorded for both short (Fritsch-Yelle et al., 1994; Gisolf et al., 2005; Verheyden et al., 2007) and long-term (Hughson et al., 2012) spaceflight. Bedrest has also been linked to decreased arterial baroreflex (Convertino et al., 1990; Traon et al., 1997; Iwasaki et al., 2000; Hirayanagi et al., 2004).

We observed elevated standing HR following HDBR (Table 3; Figure 2), an indication of greater vagal withdrawal and cardiovascular deconditioning, which continued until R+6. This increase was global, indicating that post-bedrest neither the biological sex of the participant nor exercise impacted the outcome. Raised HR upon standing is related to reduced central blood pressure, with greater HR increases commonly observed after bedrest as a compensatory reaction to increased venous pooling in the lower limbs through a lack of enhanced vasoconstriction (Feldstein and Weder, 2012; Veronese et al., 2015; Möstl et al., 2021). Similar to our discussion of EMG, changes in SBP from baseline were used to assess intergroup effects. Post-bedrest, in response to standing, male control participants had elevated SBP whereas the female control and male exercise participants had lower SBP (Figure 2). While, neither DBP nor MAP was altered significantly from baseline across test days in any test groups it must be noted that these values were averaged prior to presyncope. Blood pressure is protected at all costs and is not a reliable early predictor of presyncope (Buszko et al., 2019). Not until cardiovascular decompensation occurs will blood pressure decrease. Elevated SBP, possibly due to greater vasoconstriction in the male participants, may partially explain the significantly lower number of presyncopal males since cerebral perfusion may have been better protected than in female participants (Buszko et al., 2019).

Our data revealed a considerable reduction in cardiac baroreflex gain after HDBR (R+0) in all studied groups as well as cardiac baroreflex FTA. These data do not support the hypothesis that the prescribed exercises during bedrest would maintain cardiac baroreflex in older persons, although the SBP data is suggestive of protective vascular effects of exercise in the male participants. Differences between the male and female participants suggest that along with vascular control there are unique sex-related cardiac baroreflex control adaptations to exercise with bedrest deconditioning.

Unlike their male counterparts, female participants had significantly reduced FTA on R+0. This is an indication that on R+0 baroreflex mediated autonomic signals to the heart were either less frequent of shorter in duration compared to pre-bedrest and to males. Furthermore, the female control participants had a significant increase in causality on R+0, where all other groups, including the female exercise group, had no notable change in causality. When gain and FTA were combined as active gain and plotted with causality this contrast was more evident (Figure 4B). While male control and male and female exercise groups showed parallel declines in active gain and causality from pre-to post-bedrest, the female control group had an increase in causality while exhibiting a similar drop in active gain. Although the number of participants was four, we can postulate on a mechanism. The female control participants had significantly lower SBP than pre-bedrest and the lowest SBP of all the groups (Table 1). This may have led to an increase in cardiac causality as compensation for BP dysregulation during standing.

We expected similar losses in the cardiac arterial baroreflex after comparable durations (~2 weeks) of inactivity from HDBR or after spaceflight. Blaber et al. (Blaber et al., 2022) presented spaceflight data of equivalent duration (8–16 days) to this bedrest study using similar analyses on 10-min stand tests pre- and post-spaceflight. The astronauts did not have a significant decrease in baroreflex gain on landing day but did have a similar significant decrease in FTA. The astronauts also had a significant decrease in causality, not seen in our participants, with the women in our control group exhibiting and increase in causality. Some of the differences that may have contributed to dissimilarity could be: 1) weightlessness and HDBR not being equal in terms of unloading of the body since HDBR only removes the gravitational gradient from the head-foot axis of the body; 2) the astronauts had a mean age was 39 ± 5 years, 20 years less than that of our participants; 4) the astronauts would have physically and mentally trained for weightless for several years prior to flight while our participants, although fit, as defined by the inclusion criteria (Supplementary Material), may have had only months to prepare for HDBR; and 5) it is likely that the astronauts may not have had opportunity to exercise as extensively as our HDBR participants in the exercise group and any biological sex-related interactions with exercise would not have been observed. Further research is needed to determine the impact of immobilization/spaceflight length, effects of biological sex, and different exercise regimes on the degree of cardiac baroreflex impairment.

Reflections on space-based exercises as countermeasures during HDBR in older persons

Flight regulations on the International Space Station mandate that all crew members on long-duration missions perform exercise, which now makes it impossible to study the consequences of no exercise on the physiological impacts of spaceflight. As a result, comparison to earlier missions (Sibonga et al., 2015) or to a period before a substantial change in hardware (English et al., 2015; Sibonga et al., 2015), such as the replacement of iRED with ARED, is the only approach to assess the efficacy of current exercise countermeasures in space. The restricted opportunity to conduct controlled intervention studies, both in space and in spaceflight analogues such as HDBR, is a substantial hurdle to developing a new exercise countermeasure (Traon et al., 2007; Hargens and Vico, 2016). An exercise training intervention study is expensive and time-consuming in space. The "SPRINT" research (Rice, 2019) conducted by NASA was a unique case of a supervised, in-flight study to assess the efficacy of high intensity, low volume exercise training regimen, which demonstrated promising results in both HDBR (Ploutz-Snyder et al., 2018) and microgravity (Goetchius et al., 2020). Even in this case, the control group was not fully deprived of physical activity and continued to perform routine ISS countermeasure exercises.

Terrestrial investigations (e.g., HDBR campaigns) are likewise expensive and complicated, albeit not as much as space research, but they provide more experimental control and allow hypotheses to be addressed more rapidly. Ground-based studies of wider scope (i.e., there is no restriction on time, frequency, or intensity/overload) has allowed for the widespread acceptance of terrestrial exercise training ideas such as continuous and interval-type aerobic exercise and high-intensity, multi-set/rep resistance training And the improvement of ISS exercise countermeasure hardware (Scott et al., 2019). The combination of aerobic, HIIT, and resistance exercises employed in this study was only partially successful in preserving the muscle-pump baroreflex even though there was significant preservation of beat-to-beat muscle activity during standing. However, given the reductions in active time and reflex causality seen in some groups, the preservation of beat-to-beat muscle activity may not have been as effective with counteracting the reduction in reflex activity and causal coupling of blood pressure to muscle contractions and heart rate changes. Given the small sample size per group, our findings may suggest that the benefits of exercise intervention differed by biological sex and that they might be if tailored to biological sex. Furthermore, the exercises prescribed in this study were ineffectual in preserving cardiac baroreflex function and additional research must be conducted to assess the interrelationship between the combinations of exercises and components of the baroreflex system while taking into consideration sex-specific physiological effects.

Only menopausal women were eligible for participation in this study. Due to ethical concerns of severe detrimental outcomes of bedrest in elderly persons, the Canadian aging and inactivity study's participant age range was 55–65. This was to limit acute and long-term impacts on health yet provide sufficient data to expand our knowledge of the effects of inactivity on the elderly. One of the most important factors associated with cardiovascular disease in both men and women is the stiffening of the arterial structure that occurs as we age. However, a sudden drop in oestrogen levels in the bloodstream could contribute to an increase in blood pressure through mechanisms that are still not fully understood, such as a direct effect on the arterial wall, activation of the renin-angiotensin system and the sympathetic nervous system. (Izumi et al., 2007; Taddei, 2009; Tikhonoff et al., 2019). All elderly women are menopausal, however not all females are menopausal in our study's age range. If we were to include both perimenopausal and menopausal women, we would have been unable to accurately compare the women in the two groups (Taddei, 2009; Tikhonoff et al., 2019); therefore, it was important to exclude women who were not menopausal. Furthermore, if we were to include perimenopausal and menopausal women, we would be unable to accurately draw conclusions based on a mix of peri- and menopausal women in the control and exercise groups (Taddei, 2009; Tikhonoff et al., 2019), and it would make it difficult to draw comparisons with the outcomes of young females who are typically involved in bed rest studies.

Presyncope

Despite declines in both muscle-pump and cardiac baroreflexes, the male participants in our study had better outcomes related to presyncope compared to the female participants. Given that the prescribed exercises (Hedge et al., 2022) in HDBR were not an effective countermeasure for preserving the cardiac baroreflex an overall comparison between the two study samples is justified. Although not the only outcome expected from the implementation of exercise, prevention of syncopal events is a high priority with hospitalized older patients as this can lead to falls, co-morbidities, and death. From a space health perspective, loss of orthostatic tolerance can have operational consequences if astronauts cannot perform mission tasks within hours or days of landing on a planetary body.

In this regard, we can look at the data from shuttle astronauts who were exposed to gravitational unloading for a similar number of days (Blaber et al., 2011; Blaber et al., 2022). The fraction of presyncopal men and women following spaceflight (2/19 men, 5/7 women) was the same as in the current bedrest study (1/10 men, 6/10 women). However different the environment experienced between the two types of participants; the physiological outcome (presyncope) was the same. Orthostatic intolerance post-spaceflight in this cohort of astronauts has been attributed to reduced adrenergic vasoconstrictor response (Fritsch-Yelle et al., 1994), impaired cerebral autoregulation (Blaber et al., 2011) and decreased cardiac baroreflex (Blaber et al., 2022). Although we did not assess cerebral autoregulation in this study, our data show decreased cardiac baroreflex and blood pressure differences between groups suggestive of reduced vasoconstrictor response. We also have additional results from the muscle-pump baroreflex which was not available from the astronauts.

To provide a better understanding of the mechanisms associated with presyncope in our participants, we reanalysed the data using presyncope—those who finished the stand test (finishers) and those who did not (non-finishers)—to delineate participants, rather than biological sex. Only one variable, SBP-EMG (muscle-pump baroreflex) causality had a presyncope-specific interaction with bedrest. Prior to bedrest both non-finishers' and finishers' muscle-pump baroreflex causality were not different (0.87 ± 0.03, 0.86 ± 0.03, respectively) (p = 0.998), however, on R+0 non-finishers' causality remained the same (0.87 ± 0.03) while finishers' causality was significantly lower (0.74 ± 0.03) (p = 0.045). Finally, on R+6, non-finishers (0.84 ± 0.03) and finishers (0.79 ± 0.03) were again not significantly different (p = 0.797).

These results may reveal a global underlying response to severe orthostatic stress that was not observed in our analyses due to sex related differences in physiology and susceptibility to post-HDBR orthostatic intolerance. In the analysis presented in the results, we focused on biological sex and the exercise intervention. As a result, the data associated with presyncope was spread over several groups, predominately female. None of the males in the control group was presyncopal and had a significantly lower causality (Table 4) than pre-bedrest. The lone presyncopal male was in the exercise group (n = 5) with an SBP-EMG causality of 0.95 which skewed the value higher. Similarly, the lone finisher in the female control group (n = 4) had a causality of 0.74. and the mean value for the three finishers in the exercise group (n = 6) was (0.71 ± 0.08).

The relatively large size of the non-finisher group compared to any of the prescribed groupings has provided a unique opportunity to explore the baroreflex mechanisms employed to prevent orthostatic hypotension and fainting. None of the variables associated with the cardiac or vascular components (Tables 1, 2) were found to distinguish between non-finishers and finishers. This would suggest that the functional contributions of these two branches of the baroreflex system were equally engaged to a similar extent during stand on any given day of measurement. Skeletal muscle contractions can enhance venous return through the pumping of blood up the veins in the leg through one-way valves. In this study we found that beat-to-beat EMG output to these muscles was reduced following bedrest (Table 1). Similarly, there were reductions in muscle-pump baroreflex FTA and gain which were blunted by exercise. However, none of these were found to be related with impending syncope indicating that the baroreflex system response, although operational, was limited in the scope to which these could be altered for preventing hypotension.

A greater muscle-pump baroreflex causality in the non-finisher group implies a tighter reflex coupling of blood pressure to skeletal muscle contractions. That is, changes in blood pressure are more closely translated to a change in muscle activity which may provide a more coordinated response to hypotension. That this was observed only in the non-finisher group could be evidence that this mechanism is one of final resort for a compromised cardiovascular system, that may have been sufficient for the finishers, but not the non-finishers. Inferential evidence for the existence of leg muscle activity being associated with hypotension and orthostatic tolerance comes from the observation of increased postural sway in persons who have orthostatic hypotension based on head-up tilt or lower body negative pressure test, but do not faint in stand tests (Claydon and Hainsworth, 2005). Astronauts, as we have examined earlier in this paper, are also susceptible to OH and have greater sway post-flight (Speers et al., 1998), while patients with autonomic failure often exhibit fidgeting leg behaviours when sitting (Cheshire, 2000).

Limitations and future work

The participants in this study were selected from a healthy older population aged 55–66 years old; however, many older people are on several medications and have substantial sarcopenia even before being placed on bedrest (Blain et al., 2016; Goswami, 2017). They are frequently confined to bed owing to acute illnesses, severe injuries, procedures, or chronic ailments. Future research should investigate how different lengths of bedrest confinement affect cardio-postural connections in elderly people. This is significant because falls and fall-related injuries are frequently caused by a change in posture (upon standing from supine or sitting (Rapp et al., 2012; Goswami, 2017; Trozic et al., 2020). Future bedrest research should also include larger sample numbers in both biological sexes due to considerable sex-related variations and interindividual variability.

Physical exercise has been highlighted as a key strategy in reducing the negative consequences of bedrest confinement (Schneider et al., 2016; Ploutz-Snyder et al., 2018); however, more research is needed to compare distinct exercise types as modified and individualized exercise countermeasures for both sexes. Furthermore, sex-related differences in this study imply that the exercises should be designed specifically for each sex, which should be investigated further. More research also should be conducted to optimize training factors such as loading frequency, workload, pace, rest duration, and particular exercise "dosage" for each individual. Cognitive training (Goswami et al., 2015), LBNP (Goswami et al., 2019), pharmaceutical intervention (Lee et al., 2020), and artificial gravity (Evans et al., 2018) are further therapies that might be examined; all these have been shown to improve the symptoms of bedrest-induced physiological deconditioning. The findings can then be utilized to create and improve effective countermeasures.

Other factors that may alter postural responses, such as visual (eyes closed during testing) and vestibular inputs, were not included in the cardio-postural model presented in this article. In future investigations, a more comprehensive model combining the aforementioned factors should be adopted and examined.

Conclusion

This study evaluated the effect of 14 days of 6-degree head-down tilt bedrest (HDBR) with or without combined lower body strength, aerobic, and high-intensity interval training (HIIT) exercise countermeasures on the muscle-pump baroreflex in older adults. Physical inactivity through bedrest reduced both cardiac and muscle-pump baroreflex activation (reduced gain and FTA) during a free-standing orthostatic challenge. The exercise intervention of upper and lower body strength, aerobic, and HIIT exercise countermeasures implemented in this first Canadian aging and inactivity study (CAIS) was not found to influence the decline in cardiac baroreflex and was only partially successful in preserving the muscle-pump baroreflex even though there was significant preservation of beat-to-beat muscle activity during standing. Further analysis into the interaction between muscle activation during exercise in relation to that during the blood pressure reflex is needed to expand our understanding of the neural coupling involved.

Data availability statement

The datasets presented in this article are not readily available because data may only be shared for the use under which it was ethically approved. Requests to access the datasets should be directed to andrew_blaber@sfu.ca.

Ethics statement

The studies involving human participants were reviewed and approved by Simon Fraser Research Ethics Board. The patients/participants provided their written informed consent to participate in this study.

Author contributions

FS: Data collection, Data analysis, Validation, Writing-original draft, Writing–review; editing. RF: Statistical data analysis, Writing–review; editing. DD: Data collection, review; editing. KT: Writing–review; editing AB: Statistical data analysis, Validation, Funding acquisition, Supervision, Writing–review; editing.

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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.

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphys.2022.943630/full#supplementary-material

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Virtual Reality "exergames": A promising countermeasure to improve motivation and restorative effects during long duration spaceflight missions

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Long duration spaceflight missions will require novel exercise systems to protect astronaut crew from the detrimental effects of microgravity exposure. The SPRINT protocol is a novel and promising exercise prescription that combines aerobic and resistive training using a flywheel device, and it was successfully employed in a 70-day bed-rest study as well as onboard the International Space Station. Our team created a VR simulation to further augment the SPRINT protocol when using a flywheel ergometer training device (the Multi-Mode Exercise Device or M-MED). The simulation aspired to maximal realism in a virtual river setting while providing real-time biometric feedback on heart rate performance to subjects. In this pilot study, five healthy, male, physically-active subjects aged 35 ± 9.0 years old underwent 2 weeks of SPRINT protocol, either with or without the VR simulation. After a 1-month washout period, subjects returned for a subsequent 2 weeks in the opposite VR condition. We measured physiological and cognitive variables of stress, performance, and well-being. While physiological effects did not suggest much difference with the VR condition over 2 weeks, metrics of motivation, affect, and mood restoration showed detectable differences, or trended toward more positive outcomes than exercise without VR. These results provide evidence that a well-designed VR "exergaming" simulation with biometric feedback could be a beneficial addition to exercise prescriptions, especially if users are exposed to isolation and confinement.

KEYWORDS

sprint protocol, HIIT (high intensity interval training), resistance exercise, aerobic exercise, biometric, exergaming

Introduction

Pushing the Frontier of human spaceflight will require ever-increasing mission durations that will, in turn, require novel and creative solutions to the bigger demands on mission resources. Physical exercise remains the primary countermeasure to mitigate the health and performance decrements in astronauts caused by exposure to altered gravity environment (Clément, 2017; Richter et al., 2017; Diaz-Artiles et al., 2019). Astronauts typically exercise for 2 hours a day, 6 days a week, when onboard the International Space Station (ISS) (Hackney et al., 2015). On ISS, astronauts enjoy a suite of exercise modalities, including a cycle ergometer, treadmill, and resistive device. Trans-lunar and planetary missions will not feature such generous volume and mass allotments for their exercise systems and therefore, these missions will require the development of a singular, more integrative device as well as highly efficient protocol prescriptions (Smitherman and Schnell, 2020). A comprehensive solution to these problems remains elusive, although VR has been suggested as a promising candidate (Solignac and Kuntz, 2015; Salamon et al., 2017).

Volume, mass, usability, ease of maintenance, and schedule constraints will ultimately inform the final design of a long-duration mission exercise system. The operational usage of the system is another aspect to consider, and it is here where novel technologies and techniques can be leveraged into the mission. Finally, given the durations involved in trans-planetary missions, it is reasonable to suggest that no single system will suffice for eliciting the positive physiological and psychological effects typically associated with long-term exercise habits. Thus, integrating elements that increase variability within the exercise system and its operation, could highly benefit crewmembers embarked on a long duration exploration mission.

The current project addresses this gap through the integration of different exercise modalities and the engagement of operational strategies intended to maximize the performance of exercise countermeasures. In particular, we leverage the SPRINT protocol, a duration/intensity-modulating exercise protocol successfully deployed in a 70-day bedrest study (Ploutz-Snyder et al., 2014, 2018) as well as onboard the ISS (English et al., 2020). The protocol features increased exercise intensity *via* high-intensity interval training, which reduces the required exercise time, thus liberating crew time for other tasks in their busy schedules. The convergence of Virtual Reality (VR) gaming with exercise, often called “exergaming,” is a promising technology to enhance well-being, enjoyment, and motivation while reducing negative stress and perceived exertion (Flores et al., 2008; Murray et al., 2016). The current investigation integrates both VR gaming and exercise using a prototype exercise device designed for the needs of astronaut crew in the spaceflight environment (Tesch et al., 2013; Cotter et al., 2015). This device is called the Multi-Mode Exercise Device (M-MED), a compact flywheel ergometer that accommodates four different exercise modes (see Figure 1): supine leg press (Panel A), prone knee flexion (B), flywheel rowing ergometry (C), and supine ankle plantarflexion (D). Additional resistance can be added as angular inertia in the latter three configurations *via* 2 kg steel plates slotted externally onto the flywheel’s main drive shaft. The capability to switch between cardiovascular and resistance training without meaningfully changing the volume and mass resource design requirements of the device marks a departure

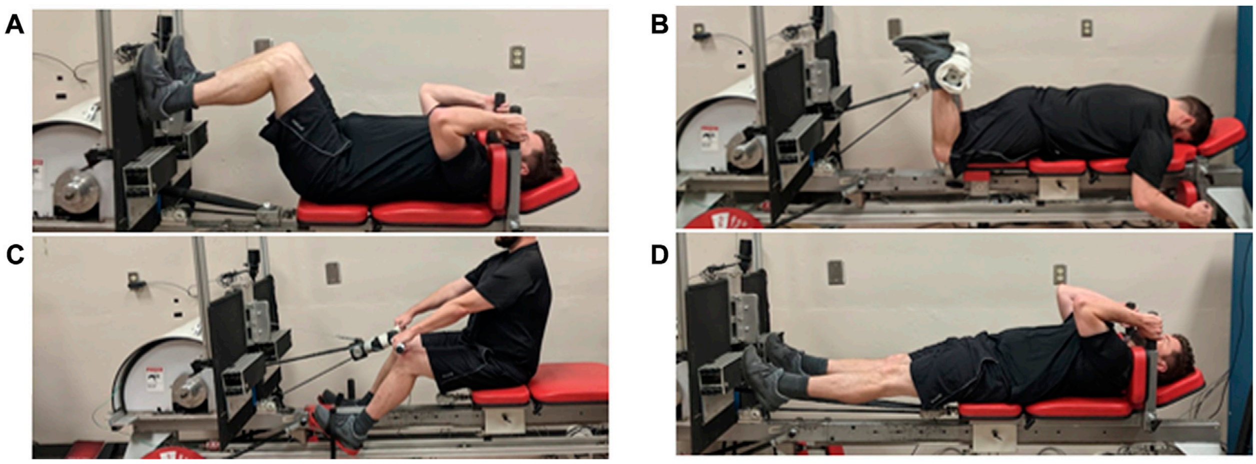

FIGURE 1
The Multi-Mode Exercise Device (M-MED) is capable of four modes of exercise. **(A)** Supine leg press. **(B)** Prone knee flexion. **(C)** Flywheel rowing ergometry. **(D)** Supine ankle plantarflexion.

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from the current state of the art onboard the ISS, where the crew utilize multiple devices to perform different exercise types, occupying most of the Tranquility module's habitation volume. The M-MED delivers resistance loads to the same muscle groups as the devices on the ISS with the exception of the canonical "push" groups (pectoralis major, triceps brachii, deltoid group, *etc.*). As these latter groups are not weight-bearing, the effect of microgravity is not as pronounced and therefore, they are less critical targets for countermeasures (de Boer et al., 2008).

Given the complexity of integrating all of these aspects for the first time, it was prudent to conduct a pilot study to validate the integration of the SPRINT protocol, the VR intervention, and the M-MED training device, and to determine the best metrics for detecting differences due to the VR intervention. We therefore selected physiological and cognitive tools broadly in order to capture these differences (if they do exist) in participants with a similar health profile to astroanuts (i.e. mid-30's, and physically fit). We expect that this preliminary work will provide a truly progressive step forward in the state-of-the-art of VR exergames countermeasures.

Materials and methods

Participants and oversight

Five male subjects were recruited according to the same criteria used in previous M-MED studies (Owerkowicz et al., 2016) (Cromwell et al., 2018): healthy subjects with a maximum oxygen uptake (VO_{2Max}) of at least 30 ml/kg/min and isokinetic knee extensor strength of at least 2 N*m/kg of bodyweight. Subject age was 35.4 ± 9.0 years old (mean ± SD), and starting Body Mass Index (BMI) was 28.7 ± 5.96. Subjects received written and verbal reviews of the study protocol and they signed their informed consent. This protocol was approved by the Texas A&M Internal Review Board on human subjects under study number IRB 2019-0471 F.

Testing modalities

A complete overview of the M-MED, the SPRINT protocol, and the VR scenario implemented in this study have been detailed previously (Keller et al., 2021).

Briefly, the M-MED, described earlier, was the exercise modality used in this study. Cardiovascular training was performed using a flywheel ergometer. Reconfiguring the M-MED device also allowed subjects to perform supine prone knee flexion, supine leg press, and supine ankle plantarflexion resistance exercises.

The SPRINT protocol required subjects to perform cardiovascular training 6 days/week (in our case, using the M-MED's rowing ergometer configuration) and resistance training 3 days/week (*via* the remaining M-MED configurations described above) (Ploutz-Snyder et al., 2018). During cardiovascular training, subjects alternated among the following two options: 1) rowing continuously for 30 min at 75% of their heart rate based on their baseline VO_{2Max}, or 2) performing high-intensity interval training (HIIT) exercise using rowing intervals of 30 s, 2 min, or 4 min at varying heart rate intensities (based on baseline VO_{2Max}). Thus, in each HIIT training session, subjects performed one of the following three protocols: a) 8*30 s at maximal effort with 15 s of active rest, b) 6*2 min at the following heart rate intensities: 70%, 80%, 90%, 100%, 90%, and 80%, with 2 min of active rest, or c) 4*4 min at 85% heart rate intensity with 3 min of active rest. Each of the HIIT protocols was performed once a week. The weekly order of the HIIT workouts was randomized across subjects, but preserved for a given subject between conditions (VR vs No-VR). Resistance training was performed on the same days as continuous rowing with a gap of at least 4 hours between both types of training. Subjects performed the following lower leg exercises: supine leg presses, prone leg curls, and supine ankle plantarflexion. Resistance loads varied nonlinearly throughout the protocol, beginning with three sets of 10 repetitions, then increasing loads until the maximum possible angular resistance allowed by the flywheel, then increasing repetitions as needed throughout the workout until muscle failure or 20 repetitions (whichever came first).

A custom-made VR simulation was developed for this investigation. The simulation was integrated with the M-MED device and deployed during cardiovascular training (both continuous rowing and HIIT training). During these sessions, subjects were seated in a virtual boat with two virtual teammates pitted against a second boat of three virtual competitors. Both boats were situated in a river scene designed to seem as realistic as possible, including natural soundscape and oar-splashing audio components (synced to visual components). Audio was also delivered *via* the Vive headset's onboard speakers, which occluded most external noises. Subjects' heart rate was monitored *via* a chest-strap (Polar H10, Polar Electro 2020) and data were streamed into the simulation and presented to the subjects *via* a biometric display in their rowing boat. This display, shown in Figure 2A, showed the real-time quantitative heart rate as well as a qualitative vertical bar indicating whether the heart rate was within the expected limits (+/− 5% of the heart rate goal). In addition, if subjects were not maintaining their heart rate goal (i.e., heart rate became too low or too high with respect to the goal), the velocity of second boat increased in real time, exceeding the velocity of the subjects' boat. Conversely, if the heart rate goal was successfully maintained, the velocity of the second boat fell just below the subjects' boat velocity (see Figure 2B). The downstream distance between the two boats was limited to 10 m to prevent an uncompetitive runaway scenario. VR simulations were not utilized during the resistance training due to the short duration of these exercise sessions.

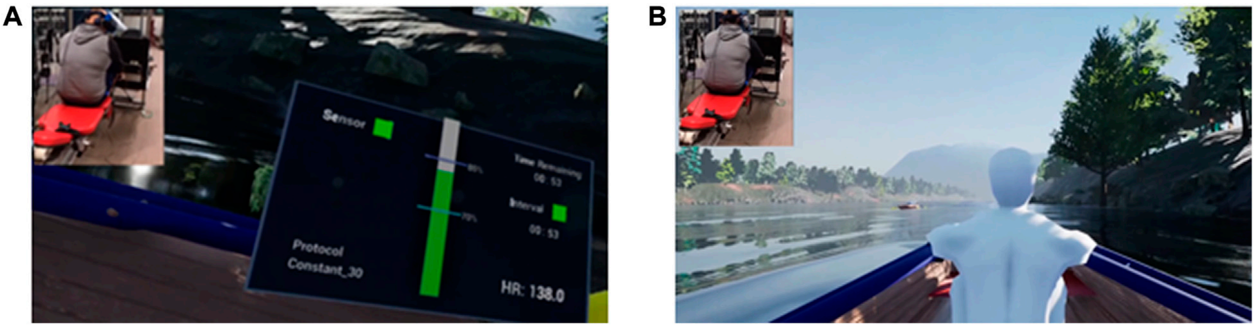

FIGURE 2
VR simulation with subject's real-time recording inset. **(A)** subject viewing real-time readouts in a virtual display, including biometric heart rate data, time remaining in the protocol, sensor connection status, and protocol information. **(B)** subject viewing the position of the other virtual boat competitors located upstream. Note the vertical green bar on the virtual display in **(A)**, indicating successful attainment of target heart rate. This state corresponds to surpassing, or "winning against", the virtual competitors as seen in **(B)**. If the subject's heart rate became too low or too high with respect to the goal, the bar turns red and the competing boat gains velocity and ultimately passes the subject, unless they were able to re-attain goal heart rate.

Experimental design

Five subjects completed a counterbalanced, within-subject study that examined the effect of our VR simulation on physiological and cognitive outcomes of a spaceflight-like exercise training scenario. Each subject first completed a 2-week SPRINT protocol using the M-MED, either with (VR condition) or without (No-VR condition) the VR simulation during the cardiovascular training sessions. After at least a wash out period of 1 month, each subject repeated the 2-week protocol in the opposite experimental condition. Two subjects started in the VR group, and three subjects started in the no-VR group. A timeline depicting a given subject's 2-week protocol is depicted in Figure 3.

The VR condition was delivered *via* an HTC Vive Pro Eye (2018, HTC Corporation, New Taipei City, Taiwan). Subjects in the No-VR group could visually monitor their real-time heart rate through an iPad (2020, Apple Inc., Cupertino, CA) placed on the M-MED that ran the proprietary Polar app showing real-time heart rate. During the No-VR condition, subjects were not permitted to listen to audio devices during exercise.

Outcome measures

A summary of the physiological and cognitive metrics analyzed and their schedule for the 2-week SPRINT protocol is given in Table 1. A more thorough explanation of each of the following metrics can be found in Keller et al. (2021), and a summary is provided below.

Pre-post measures

A broad set of physiological and cognitive measures were collected before and after each of the 2-week SPRINT conditions (VR and No-VR conditions). Physiological measures included: maximal oxygen uptake (VO_{2Max}) and maximal heart rate *via*

Day 0	C+R	HIIT	C+R	HIIT	C+R	HIIT	
"Pre" Measures	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7 (Rest)
	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	"Post" Measures

FIGURE 3
SPRINT protocol and timeline implemented in the study. Subjects exercised for 2 weeks in each one of the two experimental conditions (VR vs. No-VR). C + R indicates days of 30 min of Continuous rowing exercise at a heart rate intensity equivalent to 75% of $VO2_{Max}$. After a rest period of at least 4 hours, subjects returned to complete lower body Resistance exercises. HIIT indicates high-intensity interval training. Each of the three HIIT protocols was performed once a week, and their order was randomly selected by week and counterbalanced by subject. The order of HIIT protocols was preserved for a given subject between VR conditions.

TABLE 1 Pre-post and daily physiological and cognitive metrics employed in the 2-week SPRINT protocol. The specific days when each measure was collected are also indicated in the table. Pre-post measures were collected before and after the 2-week SPRINT protocol. Daily measures were generally collected before and after each individual exercise session. Salivary cortisol samples were only collected prior to exercise sessions. Some other daily measures, indicated with an *, were collected only after exercise sessions.

Measure	Tool	Days used
Pre-Post Measures		
Physiological		
Maximal Oxygen Uptake	Stress Test	Day 0/14
Maximal Heart Rate	Stress Test	Day 0/14
Resting Energy Expenditure	Indirect Calorimetry	Day 0/14
Blood Pressure	Arm Cuff Sphygmomanometer	Day 0/14
Body Composition	DEXA & BMI	Day 0/14
Leg Muscular Strength	Leg Press	Day 0/14
Leg Muscular Power	Leg Press	Day 0/14
Leg Muscular Endurance	Leg Press	Day 0/14
Cognitive		
Virtual Reality Value	Value of Virtual Reality (Exercise)	Day 0/14
Emotional Distress	General Health Questionnaire (28 questions)	Day 0/14
Perceived Stress	Perceived Stress Scale (14 questions)	Day 0/14
Cognitive Function	WinSCAT	Day 0/14
Motivation	Sport Motivation Scale-6	Day 0/14
Daily Measures		
Physiological		
Physical Stress	Salivary Cortisol	Days 1,3,6,8,10,13
Cognitive		
Transient Anxiety	State-Trait Anxiety Inventory (T only)	Days 1–6, 8–13
State Feeling	Feeling Scale & Felt Arousal Scale	Days 1–6, 8–13 Days 1–6, 8–13
Subjective Effort*	Rating of Perceived Exertion	Days 1–6, 8–13
Exercise Affect	Physical Activity Affect Scale	Days 1–6, 8–13
Mood Restoration*	Perceived Restorativeness Scale	Days 1–6, 8–13
Virtual Presence* (VR Only)	Spatial Presence Experience Scale	Days 1–6, 8–13

stress test, resting energy expenditure (REE) *via* indirect calorimetry, resting blood pressure (systolic and diastolic) *via* arm cuff sphygmomanometer, body composition *via* dual-x-ray absorptiometry (DEXA), and leg muscular strength, power, and endurance *via* leg press. Cognitive measures included: VR value, a measure of the bias a person may have toward VR generally, *via* the Value of Virtual Reality questionnaire (adapted for exercise; (Anderson et al., 2017)), emotional distress *via* the General Health Questionnaire (Nagyova, 2005), perceived stress *via* the Perceived Stress Scale (Cohen et al., 1983), cognitive function *via* Windows Cognitive Aptitude Test (WinSCAT) (Kane and Kay, 1997), and motivation *via* Sport Motivation Scale-6 (SMS-6) (Mallett et al., 2007). The WinSCAT includes four sub-scales, each one delivered *via* its own software to test a specific cognitive function: 1) Code Memory, a test of short-term recall; 2) Running Memory, a test of sustained attention and concentration; 3) Match to Sample, a test of visual short-term memory; and 4) Mathematical Processing, a test of verbal working memory. The SMS-6 scale includes six sub-scales derived from 24 items that can be (simply) thought of as the spectrum of an individual's motivation toward an exercise or sport, ranging from Amotivation (or a lack of motivation) to Intrinsic Motivation (or an in-born motivation independent of any external factors). A measurable transition from one sub-scale to an adjacent level or beyond over time represents an internalization or internal reorganization of the various motivational factors.

Daily measures

Another set of physiological and psychological measures was collected before and/or after each individual exercise session. To measure physical stress, two salivary cortisol samples were simultaneously collected prior to the exercise sessions on protocol days 1, 3, 6, 8, 10, and 13. Daily, cognitive metrics included transient anxiety *via* a short-form of the State-Trait Anxiety Inventory (Marteau and Bekker, 1992), state feeling regarding exercise *via*

TABLE 2 Summary of pre-post measures collected before and after the 2-week SPRINT protocol for the VR and No-VR groups (n = 5). Data were analyzed using paired sample Wilcoxon rank tests to investigate the effects of time (post v. pre) and VR condition (VR condition ($\Delta(VR) = Post_{VR} - Pre_{VR}$ vs $\Delta(No\,VR) = Post_{No\,VR} - Pre_{No\,VR}$). Data are presented as mean ± SE. Bolded items indicate $p < 0.05$.

Pre/Post measures	—	Pre	Post	Δ = post-pre	*p* Value	
					Time	VR
Physiological						
Maximal Oxygen Uptake (ml/kg/min)	VR	34.6 ± 2.3	34.6 ± 3.3	0.0 ± 1.0	0.225	**0.043**
	No-VR	35.3 ± 2.1	37.6 ± 3.4	2.3 ± 1.3	0.893	
Maximal Heart Rate (bpm)	VR	180 ± 2.5	172 ± 2.9	−8 ± 0.4	0.345	0.581
	No-VR	184 ± 4.1	174 ± 0.5	−10 ± 3.6	0.104	
Resting Energy Expenditure (kcal)	VR	1918 ± 183	1896 ± 152	−21.5 ± 31.0	0.225	0.225
	No-VR	1894 ± 180	1970 ± 235	76 ± 54.6	0.686	
Resting Systolic Blood Pressure (mmHg)	VR	119 ± 3.2	116 ± 3.7	−3.2 ± 0.5	0.356	0.138
	No-VR	118 ± 5.9	123 ± 6.1	5.2 ± 0.2	0.363	
Resting Diastolic Blood Pressure (mmHg)	VR	76 ± 2.4	74 ± 2.6	−1.4 ± 0.1	0.525	0.465
	No-VR	75 ± 3.8	78 ± 3.7	2.8 ± 0.1	0.418	
Body Fat (%)	VR	22.6 ± 3.4	22.0 ± 3.8	−0.7 ± 0.4	0.893	0.136
	No-VR	22.2 ± 3.5	22.5 ± 3.5	0.2 ± 0.0	0.225	
Leg Strength (kg)	VR	427 ± 56	461 ± 57	33.6 ± 0.7	**0.042**	0.500
	No-VR	462 ± 58	481 ± 58	19.0 ± 0.3	**0.043**	
Leg Power (W)	VR	1915 ± 260	2011 ± 292	95.6 ± 31.8	0.225	0.686
	No-VR	1910 ± 255	1995 ± 278	84.6 ± 22.7	**0.043**	
Leg Endurance (W)	VR	973 ± 132	1011 ± 157	38.2 ± 24.2	0.893	0.686
	No-VR	972 ± 166	985 ± 159	13.0 ± 7.0	0.345	
Cognitive						
Value of Virtual Reality (Exercise)	VR	26.0 ± 2.1	31.8 ± 3.2	5.8 ± 1.1	0.068	0.893
	No-VR	28.8 ± 4.2	34.4 ± 2.6	5.6 ± 1.6	0.104	
General Health Questionnaire	VR	44.8 ± 1.6	47.2 ± 2.6	2.4 ± 1.0	0.416	0.893
	No-VR	43.2 ± 1.5	44.4 ± 2.4	1.2 ± 0.9	0.715	
Perceived Stress Scale	VR	32.2 ± 3.5	34.8 ± 3.6	2.6 ± 0.1	0.465	0.715
	No-VR	33.4 ± 4.2	35.6 ± 4.7	2.2 ± 0.5	0.465	
WinSCAT Sub-scales						
Code Memory						
Reaction Time (ms)	VR	913 ± 91	979 ± 92	66.8 ± 1.0	0.500	**0.043**
	No-VR	1028 ± 116	993 ± 116	−34.4 ± 0.0	0.686	
Accuracy (%)	VR	95.4 ± 2.1	97.6 ± 1.5	2.2 ± 0.6	0.357	0.197
	No-VR	97.6 ± 1.7	94.4 ± 2.5	−3.2 ± 1.0	0.317	
Running Memory						
Reaction Time (ms)	VR	534 ± 54	580 ± 40	45.4 ± 14.4	0.465	0.225
	No-VR	561 ± 44	572 ± 51	10.2 ± 7.8	0.080	
Accuracy (%)	VR	85.8 ± 9.5	84.0 ± 8.8	−1.8 ± 0.7	1.000	0.336
	No-VR	92.2 ± 2.6	92.4 ± 1.2	0.2 ± 1.4	0.416	
Losses	VR	19.6 ± 15.7	21.4 ± 15.1	1.8 ± 0.6	0.684	0.465
	No-VR	7.6 ± 3.7	5.8 ± 1.4	−1.8 ± 2.7	0.684	
Match to Sample						
Reaction Time (ms)	VR	1466 ± 136	1713 ± 126	246.8 ± 10.8	0.893	**0.043**
	No-VR	1439 ± 69	1462 ± 94	23.2 ± 24.3	**0.043**	
Accuracy (%)	VR	98.6 ± 1.4	97.2 ± 1.7	−1.4 ± 0.3	0.581	0.357
	No-VR	96 ± 2.6	98.2 ± 1.4	2.2 ± 1.3	0.564	

(Continued on following page)

TABLE 2 (*Continued*) Summary of pre-post measures collected before and after the 2-week SPRINT protocol for the VR and No-VR groups (n = 5). Data were analyzed using paired sample Wilcoxon rank tests to investigate the effects of time (post v. pre) and VR condition (VR condition ($\Delta(VR) = Post_{VR} - Pre_{VR}$ vs $\Delta(No\,VR) = Post_{No\,VR} - Pre_{No\,VR}$). Data are presented as mean ± SE. Bolded items indicate $p < 0.05$.

Pre/Post measures	—	Pre	Post	Δ = post-pre	*p* Value	
					Time	VR
Mathematical Processing						
Reaction Time (ms)	VR	2204 ± 286	2184 ± 303	−20.4 ± 17.0	0.893	0.686
	No-VR	2170 ± 359	2175 ± 250	4.2 ± 108.9	0.500	
Accuracy (%)	VR	89.0 ± 1.0	91.0 ± 2.9	2.0 ± 1.9	0.066	0.222
	No-VR	96.0 ± 1.8	73.0 ± 16.3	-23.0 ± 14.4	0.480	
Sport Motivation Scale Sub-Scales						
Amotivation	VR	7.8 ± 3.3	4.4 ± 0.2	−3.4 ± 3.1	0.109	0.109
	No-VR	5.8 ± 1.9	7.4 ± 2.7	1.6 ± 0.8	0.257	
External Regulation	VR	9.8 ± 2.5	12.8 ± 4.80	3.0 ± 2.2	0.581	0.893
	No-VR	12.6 ± 4.5	13.0 ± 5.0	0.4 ± 0.5	1.000	
Introjected Regulation	VR	16.8 ± 2.5	18.2 ± 2.7	1.4 ± 0.3	0.461	0.786
	No-VR	14.8 ± 2.2	16.6 ± 2.0	1.8 ± 0.1	0.893	
Identified Regulation	VR	16.6 ± 3.4	18.2 ± 2.7	0.4 ± 0.9	0.917	0.345
	No-VR	14.6 ± 2.6	17.0 ± 3.1	2.4 ± 0.5	0.080	
Integrated Regulation	VR	15.0 ± 3.6	17.8 ± 3.7	2.8 ± 0.1	0.593	1.000
	No-VR	14.8 ± 8.7	15.4 ± 10.0	0.6 ± 2.2	1.000	
Intrinsic Motivation	VR	19 ± 3.9	22.0 ± 2.9	3.0 ± 1.0	0.273	1.000
	No-VR	19.8 ± 3.1	21.8 ± 2.9	2.0 ± 0.2	0.588	

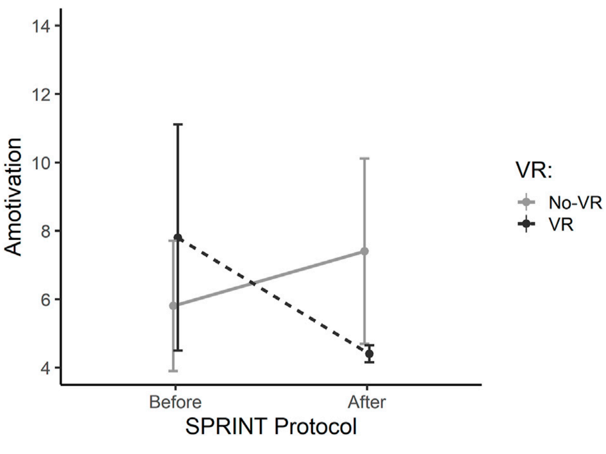

FIGURE 4
Sport Motivation Scale-6 (SMS-6) sub-scale: Amotivation, before and after the 2-week SPRINT exercise protocol for the VR (dark grey, dashed bar) and No-VR groups (light grey, solid bar) (n = 5). Results show mild trends of decreasing Amotivation for the VR group over time ($p = 0.109$). Overall differences between VR and No-VR condition in this subscale were mild ($p = 0.109$).

the Feeling Scale (Hardy and Rejeski, 2016) and the Felt Arousal Scale (Svebak and Murgatroyd, 1985), subjective effort *via* the Rating of Perceived Exertion (RPE) questionnaire (Borg, 1962), exercise affect *via* the Physical Activity Affect Scale (PAAS) (Lox et al., 2000), and mood restoration *via* the Perceived Restorativeness Scale (PRS) (Hartig et al., 1997). The PRS questionnaire includes four sub-scales described succinctly as: 1) *compatibility*, the feeling that one's goals and intentions are matched to the environment's capacity to allow them to achieve those goals; 2) *being away*, the feeling of escaping unwanted distractions external to the activity's context; 3) *fascination*, the feeling of being able to direct attention effortlessly toward contents and events in the environment; and 4) *coherence*, the feeling that environment is a calm and predictable place. Additionally, for the VR group only, virtual presence, commonly used as a metric of the immersive qualities of a VR simulation, was also measured *via* the Spatial Presence Experience Scale (SPES) (Hartmann et al., 2016). These cognitive metrics were collected before and after each individual exercise session, except for subjective effort, mood restoration, and virtual presence, which were collected after exercise sessions only.

Statistical analysis

Much of the data did not satisfy the normality assumption, most likely due to the low number of subjects. Thus, non-parametric statistical techniques were implemented.

TABLE 3 Summary of daily measures collected before and/or after each individual exercise session, for the VR and No-VR groups (n = 5). Salivary cortisol samples were simultaneously collected prior to the exercise sessions on protocol days 1, 3, 6, 8, 10, and 13. Rating of Perceived Exertion (RPE), Perceived Restoratives sub-Scales (PRS), and the Spatial Presence Experience Scale (SPES) were collected after the exercise sessions only (measures indicated with †). The rest of the scales were collected before and after each individual exercise session and these metrics are presented as delta $\Delta = Post - Pre$. Data were analyzed using a three-way, aligned-rank transform (ART) repeated measures analyses of variance with factors *VR Condition* (VR vs No-VR), *Time*, and *Workout* (HIIT vs Continuous, with HIIT training occurring on days indicated by shaded columns). Significance for main effects and interaction effects are reported. Data are presented as mean ± SE. Bolded items indicate $p < 0.05$. Italicized items indicate $0.05 < p < 0.1$.

Daily exercise effects of VR	Group	Protocol day												*p*-value				
		1	2	3	4	5	6	8	9	10	11	12	13	VR	Time	Workout	VR x workout	VR x time
Salivary Cortisol (ng/ml)	VR	48.9 ± 12.2	—	29.4 ± 5.3	—	—	35.7 ± 6.9	32.8 ± 2.1	—	48.8 ± 8.2	—	—	28.5 ± 5.4	0.193	0.114	0.947	0.738	0.776
	No-VR	53.2 ± 7.0	—	45.0 ± 12.4	—	—	42.9 ± 12.1	31.9 ± 2.6	—	63.4 ± 13.6	—	—	51.6 ± 13.2					
Δ State Trait Anxiety Score	VR	2.4 ± 1.6	1.2 ± 1.6	−0.2 ± 0.9	1.4 ± 1.2	−0.2 ± 0.9	1.0 ± 1.2	1.0 ± 0.6	−0.2 ± 1.5	1 ± 1.0	−0.4 ± 1.1	0.8 ± 0.6	1.6 ± 1.6	0.283	0.176	0.756	0.107	0.995
	No-VR	0.2 ± 1.2	1.4 ± 1.4	−1.4 ± 1.6	2.0 ± 1.1	−0.8 ± 1.0	2.5 ± 1.2	1.4 ± 1.8	0.0 ± 1.0	−1.6 ± 1.8	−0.5 ± 0.7	−0.6 ± 1.0	3.5 ± 1.6					
Δ Feeling Score	VR	0.4 ± 0.4	1.8 ± 0.4	1.6 ± 0.6	0.8 ± 0.6	1.2 ± 0.5	0.8 ± 0.6	0.8 ± 0.8	1.2 ± 0.8	0.8 ± 0.8	1.2 ± 0.5	1.0 ± 0.5	1.0 ± 0.5	0.897	0.282	0.593	0.887	0.868
	No-VR	1.6 ± 0.9	1.0 ± 0.8	1.8 ± 0.8	1.4 ± 0.7	1.6 ± 0.7	−0.5 ± 0.7	0.8 ± 1.1	1.4 ± 1.2	2.6 ± 1.2	1.5 ± 1.0	1.8 ± 0.8	−0.8 ± 1.2					
Δ Felt Arousal Score	VR	0.6 ± 0.2	1.2 ± 0.5	1.0 ± 0.5	1.0 ± 0.7	1.2 ± 0.6	1.2 ± 1.1	1.0 ± 0.4	1.2 ± 0.7	1.2 ± 0.6	1.4 ± 1.0	1.0 ± 0.5	1.4 ± 0.6	0.853	0.951	0.772	0.886	**0.039**
	No-VR	1.8 ± 0.6	2.0 ± 1.1	1.2 ± 0.7	2.2 ± 1.0	1.6 ± 1.0	0.5 ± 0.5	1.4 ± 1.0	1.6 ± 1.2	2.2 ± 1.1	1.8 ± 1.3	1.4 ± 0.9	0.3 ± 0.8					
Rating of Perceived Exertion (RPE)†	VR	15.2 ± 0.4	16.4 ± 0.2	15.0 ± 0.3	17.0 ± 0.6	15.0 ± 0.3	17.0 ± 0.5	14.3 ± 0.4	16.4 ± 0.5	14.8 ± 0.7	17.8 ± 0.4	15.2 ± 0.4	16.6 ± 0.7	0.155	0.962	**0.001**	0.528	0.540
	No-VR	15.2 ± 0.4	15.2 ± 0.4	15.4 ± 0.8	17.8 ± 0.5	15.0 ± 0.5	16.3 ± 1.4	15.2 ± 0.6	16.6 ± 0.4	12.4 ± 3.1	17.5 ± 0.3	15.0 ± 0.6	17.3 ± 0.6					
Physical Activity Affect Score Sub-scales																		
Δ Positive Affect	VR	0.2 ± 0.7	2.4 ± 0.9	2.4 ± 1.0	1.8 ± 1.2	0.6 ± 0.5	1.0 ± 1.3	1.0 ± 0.5	2.2 ± 0.7	2.2 ± 0.7	0.8 ± 0.7	0.6 ± 0.7	2.2 ± 1.0	0.429	*0.099*	0.661	0.363	0.292
	No-VR	2.8 ± 0.9	2.4 ± 1.1	1.8 ± 0.6	1.6 ± 1.2	1.6 ± 1.2	−1.0 ± 0.9	2.6 ± 1.4	2.0 ± 1.1	4.0 ± 1.4	2.25 ± 2.6	1.6 ± 1.4	−1.0 ± 2.6					
Δ Negative Affect	VR	−0.2 ± 0.2	−0.2 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	−1.0 ± 0.9	−0.4 ± 0.5	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.2	*0.083*	0.506	*0.087*	**0.026**	**0.009**
	No-VR	−0.6 ± 0.9	0.6 ± 0.4	−1.2 ± 0.8	0.4 ± 0.5	−0.2 ± 0.3	−0.25 ± 0.3	0.2 ± 0.2	0.2 ± 0.2	0.0 ± 0.0	0.5 ± 0.5	−0.2 ± 0.2	1.0 ± 0.7					
Δ Fatigue	VR	2.8 ± 1.7	1.4 ± 1.2	−0.4 ± 0.4	0.4 ± 0.2	0.8 ± 0.6	2.4 ± 1.4	0.3 ± 0.2	0.8 ± 0.6	1.4 ± 0.7	1.6 ± 0.8	0.6 ± 0.7	0.8 ± 0.7	*0.076*	0.591	0.491	**0.015**	0.134
	No-VR	0.4 ± 1.0	2.4 ± 1.7	1.2 ± 1.2	2.0 ± 0.6	0.8 ± 0.9	2.0 ± 1.1	1.0 ± 0.8	1.2 ± 0.8	1.0 ± 0.3	2.25 ± 1.0	0.4 ± 0.4	3.5 ± 0.9					
Δ Tranquility	VR	−1.4 ± 1.8	−1.0 ± 0.6	0.0 ± 0.6	−0.6 ± 0.9	0.2 ± 0.7	0.2 ± 1.7	−1.0 ± 1.0	−1.4 ± 0.8	−1.2 ± 1.0	0.0 ± 1.1	−1.0 ± 1.3	−2.0 ± 1.4	0.216	0.823	0.794	0.690	0.125
	No-VR	−1.2 ± 0.9	−1.2 ± 1.2	0.4 ± 1.1	−1.6 ± 0.8	0.8 ± 0.7	0.3 ± 0.6	−1.0 ± 1.4	0.6 ± 0.9	1.2 ± 2.1	0.8 ± 0.8	0.2 ± 0.6	0.0 ± 1.4					
Perceived Restorativeness Sub-scales (PRS)†																		
Compatibility	VR	38.4 ± 8.4	38.0 ± 8.1	40.8 ± 8.0	42.2 ± 9.0	39.8 ± 8.5	41.6 ± 8.6	37.8 ± 8.3	43.0 ± 7.5	44.6 ± 8.0	42.6 ± 8.2	44.2 ± 8.1	46.6 ± 7.0	**0.050**	0.823	0.794	0.346	0.527
	No-VR	36.8 ± 4.5	35.0 ± 4.9	39.4 ± 4.8	36.2 ± 4.7	33.8 ± 5.3	29.3 ± 3.8	36.4 ± 5.7	35.2 ± 6.0	30.4 ± 9.5	39.0 ± 6.5	38.2 ± 5.8	35.0 ± 4.3					
Being Away	VR	23.4 ± 3.6	24.0 ± 4.1	23.6 ± 4.3	24.6 ± 5.3	25.2 ± 4.9	25.8 ± 5.3	22.0 ± 5.3	26.2 ± 4.4	26.8 ± 4.2	26.2 ± 4.4	26.4 ± 4.5	26.2 ± 4.6	0.114	0.942	0.832	0.686	0.181
	No-VR	26.2 ± 3.4	26.2 ± 3.5	24.2 ± 3.7	23.4 ± 4.2	24.4 ± 3.9	21.75 ± 5.0	23.4 ± 4.0	23.0 ± 4.5	16.4 ± 5.3	20.5 ± 4.6	23.8 ± 4.5	19.5 ± 4.9					
Fascination	VR	37.2 ± 5.2	38.0 ± 5.9	38.0 ± 6.4	37.6 ± 7.5	36.8 ± 7.7	35.4 ± 7.1	33.5 ± 7.4	38.6 ± 7.4	37.4 ± 7.0	38.4 ± 7.3	37.6 ± 7.2	39.0 ± 8.2	**< 0.001**	0.959	0.966	0.905	0.991
	No-VR	28.8 ± 3.8	27.4 ± 4.6	30.0 ± 6.1	27.4 ± 5.5	26.8 ± 6.1	23.8 ± 4.9	26.2 ± 5.7	26.8 ± 6.2	20.4 ± 8.4	27.0 ± 9.6	28.6 ± 6.3	24.3 ± 5.7					
Coherence	VR	27.2 ± 0.8	28.0 ± 0.0	28.0 ± 0.0	27.8 ± 0.2	27.8 ± 0.2	27.6 ± 0.5	27.8 ± 0.2	27.8 ± 0.2	27.6 ± 0.4	27.8 ± 0.2	27.6 ± 0.4	27.8 ± 0.2	**< 0.001**	0.264	0.385	0.292	0.130
	No-VR	25.6 ± 1.7	25.6 ± 1.6	25.6 ± 1.6	25.0 ± 1.9	24.8 ± 1.9	26.0 ± 1.7	25.2 ± 2.6	25.2 ± 2.6	19.4 ± 5.1	24.0 ± 2.5	25.4 ± 1.9	25.3 ± 2.8					
Spatial Presence Experience Sub-Scales (SPES)†																		
Self-Location	VR	10.6 ± 0.6	11 ± 0.6	10.2 ± 1.2	10.0 ± 1.3	10.0 ± 1.3	10.0 ± 1.3	9.75 ± 1.5	10.0 ± 1.3	10.6 ± 1.2	10.4 ± 1.2	10.4 ± 1.2	10.6 ± 1.0	N/A	0.614	0.999	N/A	N/A
Possible Actions	VR	9.0 ± 0.9	9.4 ± 1.2	9.6 ± 1.3	9.0 ± 1.3	9.4 ± 1.2	10.0 ± 1.5	9.5 ± 1.4	8.2 ± 1.6	9.4 ± 1.4	9.2 ± 1.5	9.6 ± 1.5	9.6 ± 1.4	N/A	0.628	0.999	N/A	N/A

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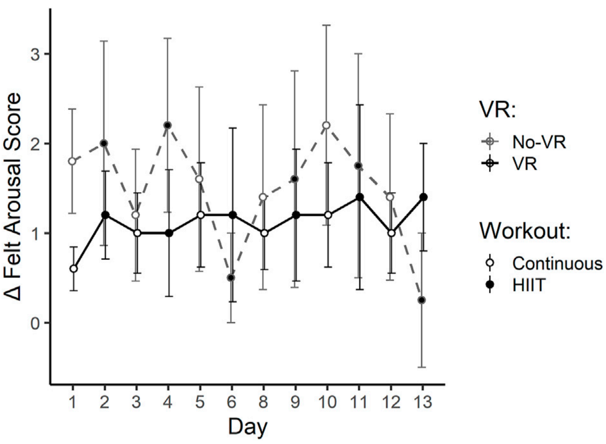

FIGURE 5
Daily scores for Δ Felt Arousal collected before and after each individual exercise session (Δ = (***Post*** – ***Pre***)) throughout the 2-week SPRINT exercise protocol, for the VR group (black, solid line) and No-VR group (grey, dashed line) (n = 5). Open symbols indicate 30-min of continuous workouts (rowing exercise at a heart rate intensity equivalent to 75% of $VO2_{Max}$), and closed symbols indicate high-intensity interval training (HIIT) workouts. Statistical testing did not show a significant effect of *VR Condition*. However, there was a significant interaction between *VR Condition x Time* (p = 0.039), indicating decreasing Felt Arousal Scores in the No-VR group over time. Data are presented as mean ± SE.

Pre-Post-measures were examined using paired samples Wilcoxon rank tests to investigate the effects of *time* (i.e., post vs. pre) and *VR condition* ($\Delta(VR) = Post_{VR} - Pre_{VR}$ vs $\Delta(NoVR) = Post_{NoVR} - Pre_{NoVR}$).

Daily measures and salivary cortisol were analyzed *via* a three-way, aligned-rank transform (ART) repeated measures analyses of variance to determine factor and interaction effects (Wobbrock et al., 2011). Factors studied includes *VR condition* (VR vs No-VR), *time*, and *workout* (HIIT vs Continuous 30 min of exercise). When measures were collected before and after an exercise session (i.e., transient anxiety, state feeling, and exercise affect), the delta between the two ($\Delta = Post - Pre$) was considered the metric of interest.

Data are presented as mean ± SE. A two-sided alpha level of 0.05 was chosen *a priori* for all statistical tests. Statistics were conducted using R Version 4.1.0 (2022, R Foundation for Statistical Computing, Vienna, Austria).

Results

Pre-post measures

Table 2 summarizes the results of the pre-post physiological and cognitive measures. Effects of the 2-week protocol on pre-post physiological measures were mostly similar within and between the VR and No-VR groups throughout the protocol. ΔVO_{2Max} was significantly higher in the No-VR group with respect to the VR group (p = 0.043). Leg press strength increased over time across both groups (VR: p = 0.042, No-VR: p = 0.043). A significant effect of time indicated increased muscular power in the No-VR group (p = 0.043). No other significant differences were detected for *time* or *VR condition* for maximal heart rate, REE, blood pressure, or body composition.

Results of the WinSCAT testing showed that Code Memory reaction time increased (not significantly) for the VR group and decreased (not significantly) for the No-VR group, and these pre-post changes between VR groups were statistically different (p = 0.043). In addition, both VR groups became slower in the Match to Sample reaction time metric, but only the No-VR group reaction time significantly increased (p = 0.043). These pre-post changes in Match to Sample reaction time between VR groups were also statistically different (p = 0.043). Mathematical Processing accuracy trended upward over time in the VR group (p = 0.066). The Sport Motivation subscale Amotivation is shown in Figure 4. Results show mild trends of decreasing Amotivation for the VR group (p = 0.109). Overall differences between VR and No-VR condition in this subscale were mild (p = 0.109). No other metrics of cognition approached significance.

Daily measures

Table 3 summarizes the results of the daily measures. Significant physical stress changes over time, between VR groups, or between workouts as determined by salivary cortisol could not be determined. Felt Arousal was not found to be significantly different with respect to the three main factors investigated (*VR Condition*, *Time*, and *Workout*), although the *VR Condition* × *Time* interaction was significant (p = 0.039), showing decreasing scores in the No-VR group (see Figure 5).

Concerning subjective effort, an overall effect of *Workout* indicated that HIIT training elicited significantly higher RPE scores than Continuous training (p < 0.001), independently of the *VR Condition* or *Time*.

Physical Activity Affect Scale (PAAS) subscales, shown in Figure 6, did not revealed significant overall effects of *VR Condition*, *Time*, or *Workout*, although the delta ((post–pre) exercise session) for Negative Affect and Fatigue were generally higher in the No-VR group with respect to the VR group (Δ Negative Affect p = 0.083; Δ Fatigue p = 0.076). In the No-VR group, the interaction of *VR Condition* and *Time* was significant for *Δ* Negative Affect (p = 0.009), which over time, generally increased compared to the VR group. In addition, *Δ* Negative Affect (p = 0.026) and *Δ* Fatigue (p = 0.015) also showed a significant *VR Condition* × *Workout* interaction effect, indicating significantly higher No-VR scores during HIIT training.

Perceived Restorativeness Scale (PRS) sub-scales, shown in Figure 7, showed significantly higher scores in the VR group

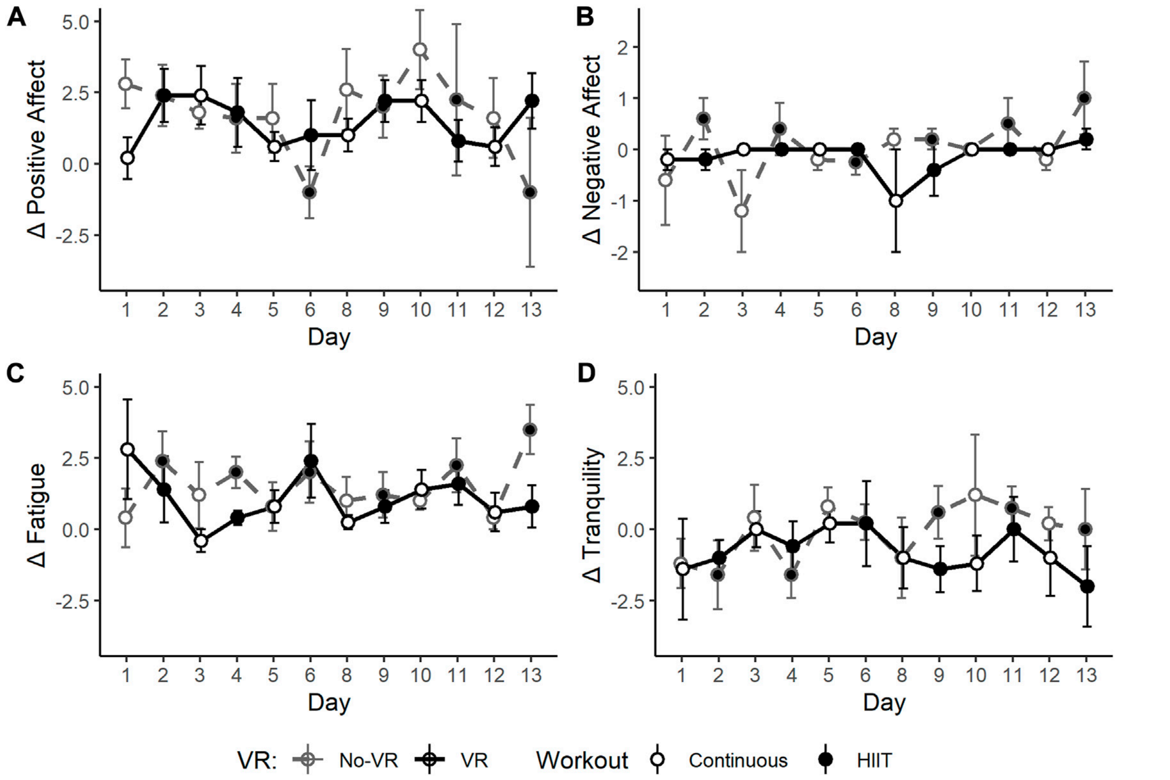

FIGURE 6
Daily scores for the Physical Activity Affect Scale (PAAS) sub-scales: Δ Positive Affect **(A)**, Δ Negative Affect **(B)**, Δ Fatigue **(C)**, and Δ Tranquility **(D)**, collected before and after each individual exercise session (Δ = (***Post – Pre***)) throughout the 2-week SPRINT exercise protocol, for the VR group (black, solid line) and No-VR group (grey, dashed line) (n = 5). Open symbols indicate 30-min of continuous workouts (rowing exercise at a heart rate intensity equivalent to 75% of $VO2_{Max}$), and closed symbols indicate high-intensity interval training (HIIT) workouts. While not statistically significant, Δ Negative Affect and Δ Fatigue were generally higher in the No-VR group with respect to the VR group (Δ Negative Affect $p = 0.083$; Δ Fatigue $p = 0.076$). For Δ Negative Affect, the interaction effects between *VR Condition* x *Time* were significant for the No-VR group ($p = 0.009$), which showed an increase in Δ Negative Affect over time. In addition, interaction effects of *VR Condition* x *Workout* were also significant for Δ Negative Affect ($p = 0.026$) and Δ Fatigue ($p = 0.015$), indicating significantly higher No-VR scores on HIIT training days. Data are presented as mean ± SE.

compared to the No-VR group in two of the four subscales: Fascination ($p < 0.001$) and Coherence ($p < 0.001$). Scores of the sub-scale Compatibility scores in the VR condition were also higher than in the No- VR condition, but they were just marginally significant ($p = 0.050$).

Overall means for Spatial Presence subscales (VR group only) for Self-Location (SL) (10.3 ± 0.36) and Possible Actions (PA) (9.3 ± 0.45) remained stable over time ($p = 0.614$, and 0.628, respectively), and were not detectably different between the Continuous (SL = 2.2 ± 0.34; PA = 2.54 ± 0.022) and HIIT (SL = 2.24 ± 0.41; PA = 2.78 ± 0.61) training days (SL $p = 0.999$; PA $p = 0.999$).

Discussion

This pilot study analyzed the effect of VR exergaming on physiological and cognitive performance metrics of a male astronaut-like population during a 2-week, spaceflight-validated, exercise countermeasure protocol on a prototype exercise device designed for the space environment. The successful deployment and integration of the VR condition demonstrated interesting trends in cognitive measures. These included improvements in metrics of mood restoration and exercise affect. Positive trends were also seen in mathematical processing accuracy, visual short-term memory (Match to Sample) reaction times, felt arousal, and reduced amotivation when compared to the identical exercise protocol in the same subjects without VR. However, trends in VO_{2Max} and in metrics of short-term recall (Code Memory) suggest that the VR condition may not have improved performance as much as the No-VR condition. Physiological effects of *Time* and *VR Condition* were minimal, but this was not unexpected given the short duration of the study.

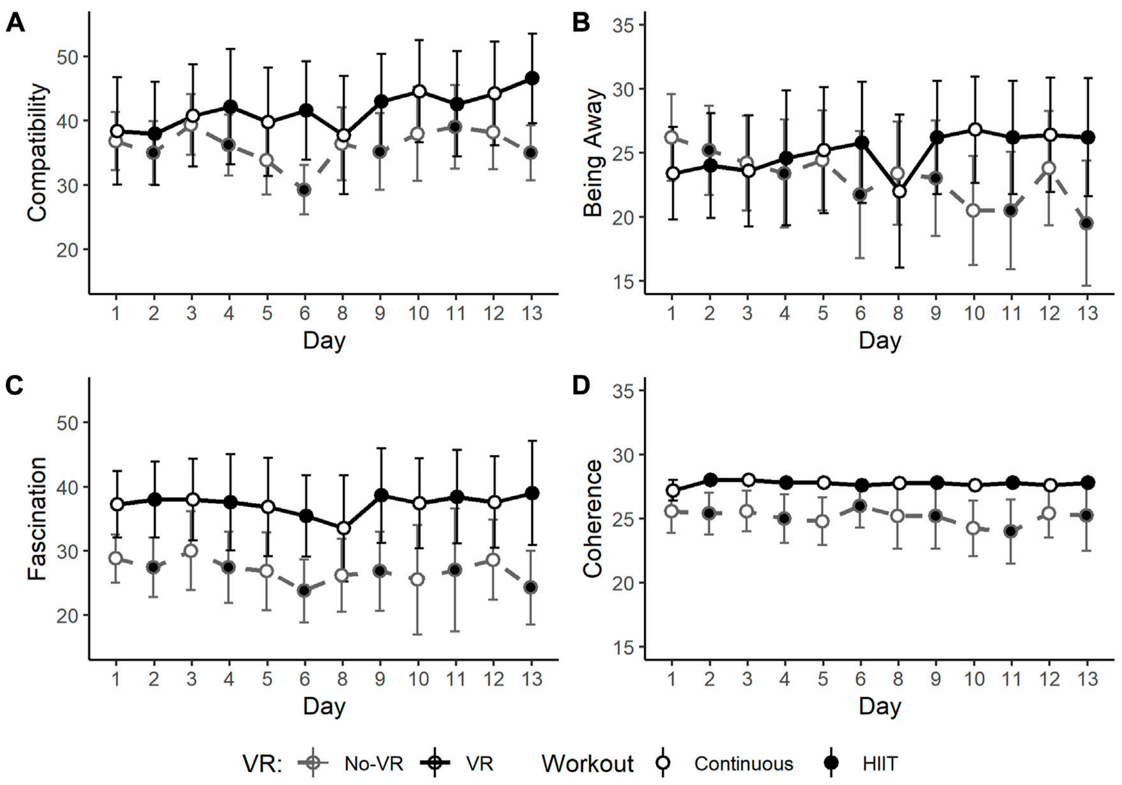

FIGURE 7
Daily scores for Perceived Restorativeness Scale (PRS) sub-scales: Compatibility **(A)**, Being Away **(B)**, Fascination **(C)**, and Coherence **(D)**, collected daily after each individual exercise session throughout the 2-week SPRINT exercise protocol, for the VR group (black, solid line) and No-VR group (grey, dashed line) (n = 5). Open symbols indicate 30-min of continuous workouts (rowing exercise at a heart rate intensity equivalent to 75% of $VO2_{Max}$), and closed symbols indicate high-intensity interval training (HIIT) workouts. Fascination and Coherence scores were statistically higher in the VR group compared to the No-VR group ($p < 0.001$ for both). Compatibility scores were also marginally significantly higher in the VR group compared to the No-VR group ($p = 0.050$). Data are presented as mean ± SE.

These pilot results support growing evidence for the efficacy of VR in exercise performance outcomes (De La Torre et al., 2018). VR exergaming is an emerging field in recreational activity (Finkelstein et al., 2010) and rehabilitation (Asadzadeh et al., 2021), with promising outcomes in physical as well as mental and social health (Loos and Kaufman, 2018). Strong SPES sub-scale scores indicate high immersive qualities of the custom VR condition created for this pilot study. In a 2017 systematic review, Matallaoui et al. showed that few exergaming studies integrated gamification design principles, favoring instead a basic shift from button-based inputs to movement-based inputs alone, and that effects of exergaming could be enhanced if those design principles were considered (Matallaoui et al., 2017). The design implemented in the present study includes several such principles: an in-game avatar, continual progress toward a known goal, and virtual competition. Previously established restorative effects of exercise, particularly when considered in light of the isolation and confinement inherent to long-duration spaceflight simulations and the COVID-19 pandemic, have been further elevated with the integration of VR (Choukér and Stahn, 2020; van Cutsem et al., 2022). Trends seen here in amotivation, felt arousal, physical activity affect, and restorativeness suggest that the VR condition generally augments the effects of exercise alone on these metrics, a finding that supports a 2021 systematic review on the use of VR in exercise rehabilitation (Asadzadeh et al., 2021). Further, the slope of trends seen in Figures 4–7 suggest that a longer-term study with more subjects may yield more significant results, like those seen in a previous VR vs No-VR running study (Neumann and Moffitt, 2018). It is also possible that some subjects found the presence of a competing boat to be a motivational factor, even if the boat was virtual, an effect also seen previously in VR (Murray et al., 2016; Parton and Neumann, 2019). VR alone (absent exercise) has been demonstrated to elicit restorative effects (Anderson et al., 2017), therefore the possibility of enhancing this effect with exercise seems worthy of further investigation.

It is particularly noteworthy that despite the small sample size, clear trends emerged in the efficacy of the VR condition in the more intense modes of exercise. The SPRINT protocol mandates high-intensity interval training on alternating days, and it was on these days (closed symbols in Figures 5–7) that many of the strongest differences manifested between the VR and No-VR groups, such as lower negative affect and fatigue. This is similar to other findings of VR effects in high-intensity modes (Barathi et al., 2018; Farrow et al., 2019).

These findings are also relevant to the burgeoning industry of exergaming. To tackle the issue rising global obesity, or even to provide viable ways of escaping isolation and confinement in a future pandemic, strategies for making physical activity more accessible, engaging, and rewarding may be possible through VR. To our knowledge, no market solution currently available integrates real-time biometric data with exergaming performance such as what was studied here. This gap represents a possible innovation that merits further investigation. Future work should include alternate natural landscapes for users to choose from, and additional, optional, competitive elements such as competing against personal records and/or against the performance of other users.

Limitations

While the M-MED is a robust and versatile platform for exactly this kind of study, it was never designed to be flown to space and therefore lacks many of the mechanical constraints required by later, more modern, designs such as the MED-2 (Downs et al., 2017). The novel nature of the hardware integration pipeline from chest-strap monitor, to bespoke software integration, proprietary software integration, and finally wired headset display, suffered from reliability and ergometric setbacks. Namely, the setup and implementation of the data stream could be simplified, and the VR headset would greatly benefit from a wireless adapter to prevent the cord from interrupting user movement. The VR simulation itself, while very promising, was limited to a single river competition setting for use in the rowing configuration of the M-MED.

While metrics of exercise performance and body composition were examined, no specific muscles were analyzed. Long-term unloading of weight-bearing muscles elicits pronounced atrophy, as noted in the introduction, and exercise countermeasures to this effect should ensure these muscles are protected.

Finally, it is clear that the study could benefit from greater statistical power. However, initial human testing started in the spring of 2020 halted due to the global COVID-19 pandemic. Budgetary constraints prevented the complete replacement of previous data, which therefore precluded many of the correlative and modeling calculations we had planned to run on sub-groups such as VR biases and personality types. It should also be noted that subjects who began in the VR group only to repeat the protocol later without VR may have experienced attitudes toward the protocol, which were not captured, nor offset, by any attitude changes in subjects who started without VR. In effect, a hypothetically disappointed subject's cognitive scores may not have been offset by an excited subject's cognitive scores who received the VR condition in the opposite order. A larger study will need to be conducted to detect such an effect, if it exists.

Conclusion

The intervention of real-time biometrically-integrated VR with spaceflight exercise protocols was sufficient to elicit detectable differences between VR and No-VR groups in $VO2_{Max}$ and several cognitive metrics. Spatial Presence Experience Scale (SPES) scores of the VR environment began high and maintained these performance values throughout the 2-week protocol, indicating a strong immersive quality into the VR scenario. Differences in outcomes generally favored the VR condition, including activity Negative Affect, Fatigue, Compatibility, Fascination and Coherence, although $VO2_{Max}$ and some cognitive measures (Code Memory, and Match-to-Sample reaction times) were more favorable in the No-VR group. The VR condition showed further differences in cognitive metrics of Negative Affect, Fatigue, and Being Away when comparing high- to moderate-intensity cardiovascular workouts. Further trends in motivation subscales, exercise affect subscales, felt arousal, and restorativeness subscales were noteworthy but not significantly different in this pilot study. Carrying on this work with a longer timeline and in more limiting environments like those enforced during social lockdowns, long-term bedrest, or space exploration, may demonstrate more robust effects. These conclusions merit a more thorough evaluation through future studies of long-term isolation and confinement interventions, as well as investigations into the effects of VR exergaming on exercise outcomes in the general population.

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 Texas A&M University Human Subjects Protection Program. The patients/participants provided their written informed consent to participate in this study.

Author contributions

AD-A is the principle investigator of this project. NK is the lead author and worked alongside RW on the statistics and manuscript. NM, AJ, and CD are advised by GC and together this team developed the VR software. LP-S created the SPRINT protocol. LP-S, GT, and MS-M lent expertise to the development of the experimental design and 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.

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4 days in dry immersion increases arterial wall response to ultrasound wave as measured using radio-frequency signal, comparison with spaceflight data

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Recent studies have reported a significant increase in common carotid artery (CCA) intima media thickness, wall stiffness and reflectivity to ultrasound, in astronauts, after six months of spaceflight. The hypothesis was that 4 days in dry immersion (subjects under bags of water) will be sufficient to change the CCA wall reflectivity to ultrasound similar to what observed after spaceflight. Such response would be quantified using the amplitude of the ultrasound signal returned to the probe by the target concerned. [coefficient of signal return (Rs)]. The Rs for anterior and posterior CCA wall, sternocleidomastoid muscle, intima layer and CCA lumen were calculated from the ultrasound radio frequency (RF) data displayed along each echographic line. After four days of DI, Rs increased in the CCA posterior wall (+15% +/- 10 from pre DI, $p < 0.05$), while no significant change was observed in the other targets. The observed increase in Rs with DI was approximately half compared to what was observed after six months of space flight (+34% +/- 14). This difference may be explained by dose response (dry immersion only four days in duration). As a marker of tissue-level physical changes, Rs provide complimentary information alongside previously observed CCA wall thickness and stiffness.

KEYWORDS

RF signal, carotid, dry immersion, ultrasound, arterial wall

Introduction

Recent studies have reported a significant increase in common carotid artery intima media thickness and wall stiffness in astronauts, during and after six months of spaceflight (Hughson et al., 2016; Arbeille et al., 2017). These morphological and dynamic changes were assessed by ultrasound using B mode echography and time motion mode (M mode). The same measures (increased intima media thickness or wall stiffness) were also observed in the central circulation (aorta) and peripheral limb arteries (superficial femoral artery) (Arbeille et al., 2017), as well as faster pulse arrival time (Baevsky et al., 2007; Hughson et al., 2016).

In the absence of direct human histological data, it was hypothesized that an increase in thickness and stiffness may be related to (a) blood flow and hydrostatic pressure redistribution induced by zero gravity, (b) cardio-metabolic dysregulation (Hughson et al., 2016; Rudwill et al., 2018) induced, in turn, by the reduction of physical activity (Fraser et al., 2012), an inappropriate nutritional regimen (Sandal et al., 2020) and/or environmental and psychological stress (Strollo et al., 2018; Muid et al., 2019). Further, the increase in thickness and or stiffness may be associated with an architectural or content change in the structure of the vessel wall which could change its capacity to reflect ultrasound. The signal return coefficient (Rs) calculated from the native radio frequency (RF) ultrasound signal provides complementary direct information on wall response (components ?), in the absence of a tissue biopsy.

Due to technical limitations described below, traditional B mode and M mode cannot be used to accurately measure "brightness" or reflectance information, particularly on targets located at different depths along the image.

In commercial devices, brightness is problematic to measure directly. The energy reflected by each target is automatically gain-compensated by the software algorithm to artificially display targets at different depths with equal brightness, for example the near and far walls of the carotid artery. In addition, the sonographer can manually adjust the brightness/gain, confounding comparisons across timepoints. In contrast to these traditional, processed modalities, the coefficient of signal return (Rs) is a brightness intensity measure with no post-processing. Rs is different because it sidesteps the processing algorithms in the manufacturer's software. It is calculated directly from the native ultrasound signal returned by each target intersected by the ultrasound scan line. Thus the coefficient of signal return can quantify the reflectance of various targets (vessel wall, muscle.) independently which allow to evidence physical transformation (structure content) of each of these (Arbeille et al., 2021). The RF signal has been used previously to measure tissue displacement in the carotid artery walls (Palombo et al., 2012), brain tissue (Rytis et al., 2020) and tissue microstructure after being heated by focused ultrasound (Mehdi et al., 2007; Maleke and Konofagou, 2008). Additionally, the native RF data have been used to describe a relationship between increased stiffness and the reduction in micro-displacement inside the tissue (Maleke and Konofagou, 2008).

Human spaceflight analogs have been used to partially reproduce the effects of spaceflight on various physiological systems. Long term head down bedrest induces a fluid shift towards the head and reduces physical activity, while confinement on earth in small habitats does not induce this cephalad fluid shift, but instead induces similar stress along with inactivity. These analogs show some of the arterial changes observed in spaceflight (Arbeille et al., 2014; Carlo et al., 2015; David et al., 2017; Strollo et al., 2018). Lastly, the dry immersion analog, which consists of having subjects lie under bags of water, invokes a fluid shift due to the pressure of the water on the legs and abdomen. Dry immersion is also an proposed analog for stress and complete inactivity (David et al., 2017; Linossier et al., 2017; Greaves et al., 2021). After spaceflight, the amount of ultrasound energy returned to the probe by the arterial walls (Rs) was significantly higher than preflight. This raised the hypothesis that particles that are highly reflective to ultrasound may be the reason, knowing that calcium is released by the bones in microgravity and may have subsequently entered the vessel wall. Knowing that an important bone calcium occurs after only four days in dry immersion (Linossier et al., 2017), we aimed to measure the Rs after 4 days of immersion.

In this study, subjects underwent four-days of dry immersion. We hypothesized that a) common artery vessel wall coefficient of signal return (Rs) would increase due to structural/content changes to the arterial wall, b) this change would be attenuated compared to six months of spaceflight and c) surrounding tissue targets' coefficient of signal return (Rs) would not increase.

Our objective was to collect and process the RF signal along dedicated, B mode ultrasound scan lines, select the location of each scan line from the B mode image and calculate the coefficient of signal return (Rs) of each of the structures intersected by the scan line. The targets were: sternocleidomastoid muscle, anterior and posterior common carotid artery walls and their respective intima layers, and the lumen.

Data were collected from participants after four days of dry immersion (DI-4days) and compared to the results of another study performed on astronauts (postflight) collected with identical hardware and signal processing methodology (Arbeille et al., 2021).

Material and methods

Dry immersion study protocol

A total of 9 subjects were included in the study (9M, age: 34 ± 7, height: 176 ± 6 cm, weight: 74 ± 7 kg). All subjects were informed about the experimental procedures and gave their written informed consent. The experimental protocol conformed to the standards set by the Declaration of Helsinki and was approved by the local Ethics Committee (CPP Est III: 2 October 2018, n° ID RCB 2018-A01470-55) and French Health Authorities (ANSM: 13 August 2018). Clinical Trials.gov Identifier: NCT03915457. The dry immersion protocol included four days of ambulatory baseline measurements before immersion (DI-4days to DI-1day) 5 days (120 h) of dry immersion (DI-1day to DI-5days) and 2 days of ambulatory

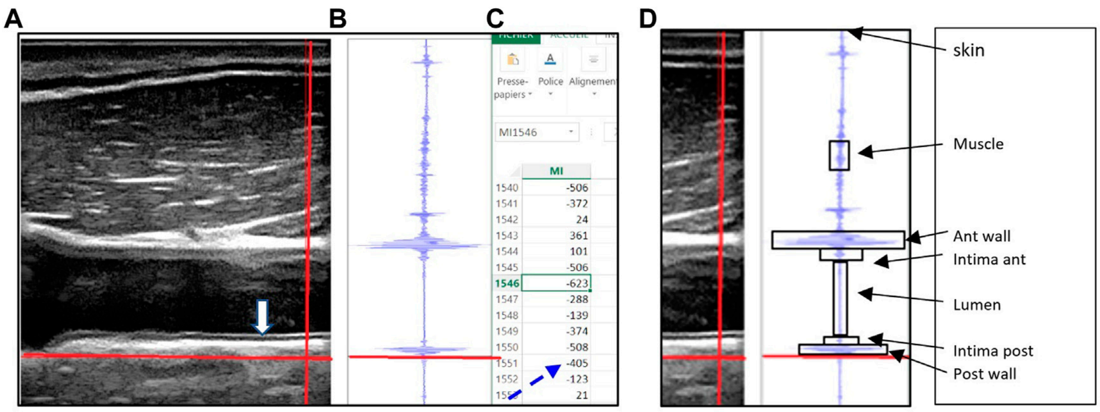

FIGURE 1
(A) B mode echo image with the movable (red) crosshairs intersecting all of the following structures: the sternocleidomastoid muscle, common carotid artery intima layers (anterior and posterior), lumen and artery wall (anterior and posterior), **(B)** the radiofrequency signal displayed graphically for points along the vertical (red) crosshair selected on the B mode image, along with the horizontal red line which allows the user to select the reflected signal value corresponding to the crosshairs' intersection point. **(C)** Spreadsheet outputs showing the actual reflected signal values, **(D)** Each target is limited by a box (arrows) which allow to identify on the Excel file all the group of cells representing the RF value reflected by the target.

recovery (R0, R+1). The DI protocol was as previously detailed in De Abreu et al, (2017). During DI, subjects remained immersed in a semi recumbent position for all activities and were continuously observed by video monitoring. Due to various measurements requiring the subjects to move out of the water tank on Day 5, the final ultrasound scans in real DI were completed on day four (DI-4days).

Spaceflight study protocol

Data from the Vascular Echo study was used to compare dry immersion to spaceflight. Eight subjects (8M) were investigated before and four days following a six month mission to the International Space Station (Arbeille et al., 2021). Crewmembers were tested first thing in the morning, caffeine restricted and prior exercise was controlled. Crew were recruited and all procedures were conducted in accordance with procedures approved by the University of Waterloo Institutional Clinical Research Ethics Board (CREC), NASA JSC Ethics Board (IRB), NASA Human Research Medical Research Board (HRMRB), European Space Agency Medical Research Board (ESA MRB) and the Japanese Space Agency Medical Research Board (JAXA MRB). Identical hardware and processing was completed as for the dry immersion dataset.

Coefficient of signal return "Rs" evaluation

The Sonoscanner echograph (Orcheo-Lite, Paris, France) was customized by the manufacturer to provide the RF data. The Sonoscanner viewer software split the screen vertically into three panels (Figure 1). The B mode image (Figure 1A) provided the user a display of the organs (Carotid) so that the vertical line used for RF output (bright thick line) can be chosen by the operator on this image. In the second panel (Figure 1B), the RF signal intensity trace of the chosen line (on the B mode) was displayed. In the right panel (Figure 1C), an interactive Excel spreadsheet was displayed, listing RF reflected signal amplitude values in a highlighted cell corresponding to the cross section of the vertical and horizontal bright line on the B mode image, as previously described in Arbeille et al, (2021).

To collect the data, the 17 MHz linear scanning probe was placed consistently by an expert sonographer directly on the dry immersion subjects and on the astronauts.

In both studies the probe had to be placed on right side of the neck, long axis orientation, cable end exactly perpendicular to the skin, with one edge in contact with collarbone. Common Carotid Artery (CCA) intima layers (anterior and posterior both) were brought into focus and the RF capture mode over the whole image was triggered.

Offline, five RF sample lines were selected where the six target structures of interest were clearly resolved (box—Figure 1D), and the embedded spreadsheet outputted. The RF signal values were squared (because $Amplitude^2$=energy) then summed over the cells (Figure 1C) corresponding to the target area (box along the RF line - Figure 1D), to calculate the energy reflected by each target structure (S target). The energy reflected by these targets were labeled: S. skin, S. muscle, S. anterior wall; S. anterior intima, S. Lumen, S. posterior intima, and S. posterior wall.

The coefficient of reflectivity R was calculated for each of the structures crossed by the ultrasound beam as the ratio between the energy "S(i)" reflected by the structure (i) by the incident energy received by the structure. The incident energy received by each structure (i) is equal to the total incident energy minus the energy reflected by the structures above the present one as previously described in Arbeille et al, (2021). One may notice that the coefficient of signal return is different from the usual "coefficient of reflection" as this one takes into account the energy reflected by the interface between 2 structures while the "coefficient of signal return" takes into account the energy reflected by the whole structure below the interface.

$$R\,muscle = \left(\frac{S\,muscle}{S\,total - S\,skin}\right)$$

$$R\,anterior\,wall = \left(\frac{S\,anterior\,wall}{S, total - S\,muscle\,to\,skin}\right)$$

$$R\,anterior\,intima = \left(\frac{S\,anterior\,intima}{S, total - S\,anterior\,wall\,to\,skin}\right)$$

$$R\,lumen = \left(\frac{S\,lumen}{S, total - S\,anterior\,intima\,to\,skin}\right)$$

$$R\,posterior\,intima = \left(\frac{S\,posterior\,intima}{S, total - S\,lumen\,to\,skin}\right)$$

$$R\,posterior\,wall = \left(\frac{S\,post\,wall}{S, total - S\,posterior\,intima\,to\,skin}\right)$$

In the present example the horizontal red line is located at the lower limit of the posterior wall (on B mode and RF trace) and the corresponding RF reflected value is 405 (dotted arrow). (Figure reproduced with permission from Ultrasound Med biol).

Statistical analysis: A 2-way RM ANOVA (Anova test function, R Studio, Netherlands) was completed to compare the mean coefficient of signal return between the two timepoints (pre DI and at 4 days in DI) among the tissue types, after testing for normality (qqplot). Pairwise t-tests were used for post-hoc comparisons between timepoints with significance at p < 0.05.

Results: After four days of dry immersion (DI-4days) the coefficient of signal return of the posterior wall (Figure 2A) was significantly higher compared to pre-immersion (64.4% +/-14 pre to 74.3% +/-16.6, p=0.03; df=8). This corresponded to a mean change of 15% +/-from pre DI. All other targets (muscle, intima layer, lumen) were unchanged (Figure 2A, Figure 3).

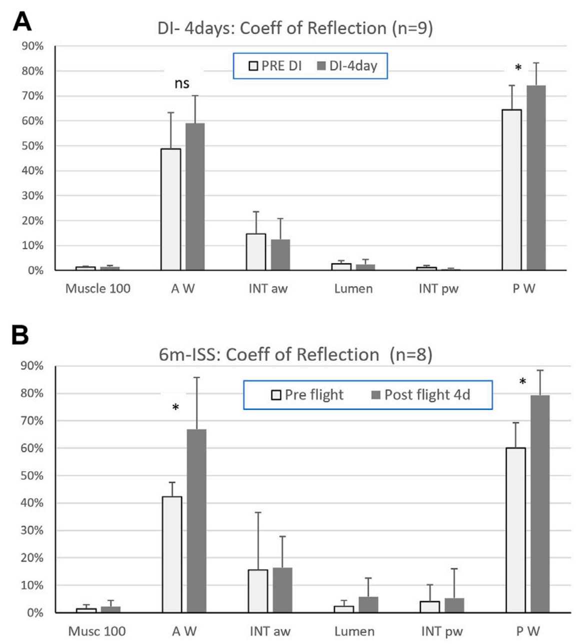

FIGURE 2
(A) Coefficient of signal return of the sternocleidomastoid muscle (Musc 100), carotid artery walls (anterior = AW, posterior = PW), carotid artery intima layers (anterior = INT aw, posterior = INT pw), and lumen, pre and after four days in dry immersion (Mean ± SD - (*) p < 0.05; n=9) and **(B)** after six months in space (6m ISS). (Mean ± SD - (*) p < 0.05; n=8). (reprinted with permission from Ultrasound Med Biol)

Discussion

After four days in DI and after six months in space, a significant increase in the posterior wall coefficient of signal return Rs was reported, but with a much lower percent increase in DI (15+/-10% in DI versus 33% +/14 postflight). Interestingly this amount of change is in the same order of magnitude as the previously observed increase in stiffness (distensibility index: 9+/-17% (Greaves et al., 2021) and also in spaceflight twice more (distensibility index: 34+/-42%) (Arbeille et al., 2017).

During DI, both carotid arterial wall return ultrasound signal amplitude and stiffness were increased to a lesser extent than after spaceflight (Hughson et al., 2016; Arbeille et al., 2017). The intima media thickness did not change in the DI subjects (Greaves et al., 2021) while it was found to increase in a majority of astronauts (>10% on 5/8) tested (Arbeille et al., 2017). These differences might be dose-related; the dry immersion was short compared to the duration of spaceflight while presenting a high degree of similarities with spaceflight condition: no physical activity, environmental stress, and a significant fluidshift towards the head.

Human histological data on carotid wall structural changes do not exist, but the macroscopic wall modification observed (thickness, stiffness.) in normal subjects in extreme environment (confinement, bedrest, spaceflight, dry immersion) resemble to those observed with aging. Several studies on the elderly population person or Diabetic patients suggest that the aged artery should be characterized by: changes in microRNA expression patterns, autophagy, smooth muscle cell migration and proliferation, collagen and elastin transformation/ proliferation and arterial calcification with progressively

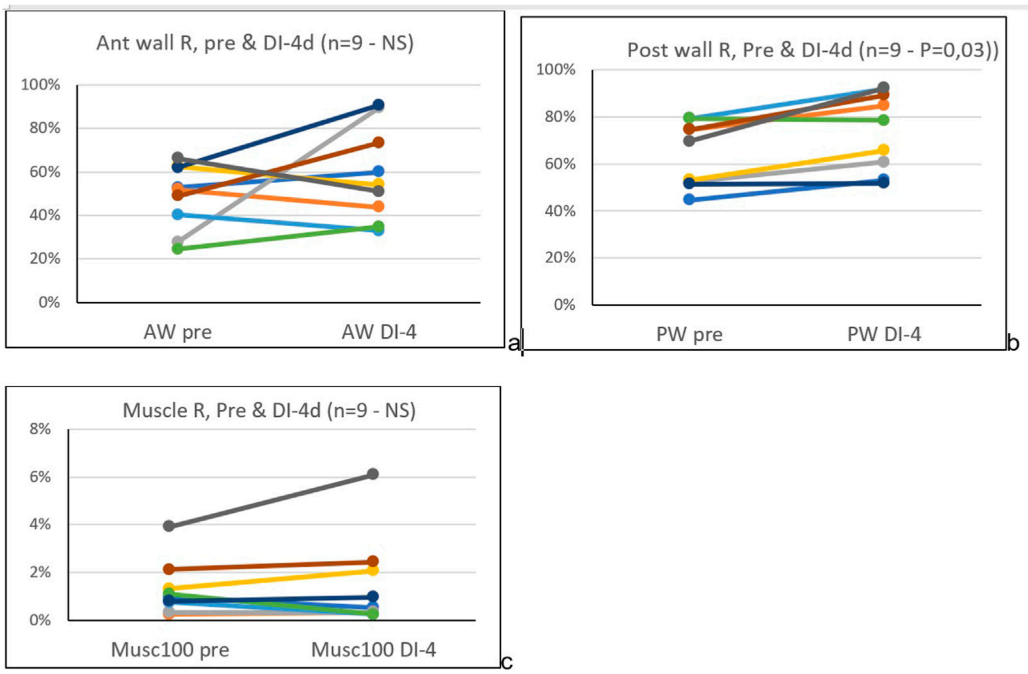

FIGURE 3
Pre and four days dry immersion, coefficient of signal return (Rs), of the common carotid artery anterior **(A)** and posterior **(B)** wall, and sternocleidomastoid muscle **(C)**, individual data.

increased mechanical vessel rigidity and stiffness (Shirwany and Zou, 2010; Tesauro et al., 2017) and reflectance to ultrasound.

Other studies suggest that vascular calcifications are actively regulated biological processes associated with crystallization of hydroxyapatite in the extracellular matrix and in cells of the media or intima of the arterial wall (Lanzer et al., 2014).

If these unknown "products" are highly reflective particles the coefficient of ultrasound signal return should increase. We speculate that these products could be calcium deposits, as increased calcium mobilized from the bone loss into the bloodstream is known to occur with spaceflight (Smith et al., 2015; Vico and Hargens, 2018). In the case of dry immersion, increased bone resorption while evidenced (Linossier et al., 2017) may not be sufficient to explain the increase in signal return coefficient (Rs). But one may notice that the significant loss in bone calcium was the only disturbance present as soon as the first 48h in DI. On the other hand, lower limb arterial calcification with vascular aging (as measured by HR-pQCT has been associated with abnormal bone microstructure and mineral density changes. Such findings suggest a possible pathophysiological link between osteoporosis and vascular calcification (Paccou et al., 2016). Additionally, DI has been shown to impact iron metabolism with increased spleen iron concentration, increased hemolysis, myolysis and serum iron concentration (Kévin et al., 2020). Thus iron deposits may also contribute to the observed increase the Rs coefficient. Without direct histological evidence, however, these processes remains speculative.

Rs represents a ratio between the ultrasound energy returned by the carotid wall and the energy of the ultrasound energy reaching it. The amount of ultrasound energy return depends on the vessel wall cell architecture and content at rest, while the stiffness index is a global mechanical parameter which measures the capacity of the vessel wall to expand during systole i.e. a parameter of function while the artery is not at rest.

The coefficient of signal return provide physiological information for individual layers in the vessel wall as it does for the six separate targets presented here. When combined with a stiffness index, together Rs and distensibility provide a powerful tool to assess both structural (artery at rest) and functional (dynamic artery) changes with spaceflight and aging.

In this paper, the target presented as "wall" is actually the media and adventitia combined and compared separately to the intima layer. For the first time, we present ultrasound data specifically on the media-adventitia, with observed changes in Rs, separately from intima, with no change. This is useful structural information to have because the traditional stiffness measures observe only the global dynamic response of the entire vessel wall to intra-vascular pressure changes. These data show that the increase in stiffness corresponds to change in the media-adventitia layer with no change in the intima.

Arterial stiffness increases with natural human aging (Gepner et al., 2014). A 15% increase in stiffness observed postflight corresponds to, on average15-20 years of aging on Earth. Increased stiffness reported during (after 15days inflight) and after six months of spaceflight (Arbeille et al., 2017) and bedrest were associated with postflight, and post bedrest altered glucose metabolism (Heer et al., 2014; Hughson et al., 2016). but the link between stiffness and cardio-metabolism is not yet clear. Moreover, the trajectory of post-flight recovery is still under evaluation, but even so, it is important to understand the process underlying such a change with real or simulated microgravity in order to develop countermeasures to prevent it during longer sojourns to the moon and Mars.

Contrary to arterial stiffness, we do not have any longitudinal data on the changes in carotid coefficient of signal return (Rs) with natural human aging. Nevertheless, the present study provides value in that it demonstrates that the Rs coefficient changes as early as four days in dry immersion, which means that the vessel wall (structure or content) can modify very quickly and that these changes can be measured by the ultrasound energy returned by the wall.

Importantly, in both studies, the carotid wall Rs coefficient changed against a background of no change in the surrounding tissues, such as the sternocleidomastoid muscle and vessel lumen. These results are consistent with the MRI investigations performed several days post flight (Mc Namara et al., 2019) and at the end of DI, which reported no significant changes to skin or muscle structure and hydric content. These consistent observations point to specificity of the coefficient of signal return as a tissue biomarker.

Study limitation: The dry immersion modality only partially mimics the spaceflight condition. a) While the water bag pressure induces a fluidshift toward the head there is still a 1G gravity applied from the head to the abdomen which partially counteracts this DI fluid shift, especially at the brain level. b) Moreover, during the first day in DI there is a major loss of fluid *via* the kidneys, the subject becomes hypovolemic and the neck/head liquid engorgement reduces significantly by the end of Day 1 (De Abreu et al., 2017). Conversely during spaceflight the neck/head liquid engorgement is present during the entire flight. c) DI does not allow for any physical activity, while during spaceflight the crewmembers have access to exercise equipment such as the T2 treadmill, C2 cycle and ARED resistance device. d) The DI subjects are highly stressed and psychlogicaly affected. e) The number of subjects is limited (n=8 in space and 9 in DI) both in flight and in DI, thus further studies are necessary to confirm the present findings. f) At last the Rs parameter should be validated *in vitro* and/or cadavers using to better understand if calcium particles are possiblyincreasing the Rs. A very basic proof-of-concept *in vitro* study was conducted using the same imaging protocol as above. 2.5g of calcium carbonate was suspended in 20 ml of water (12.5% Ca) and this "phantom" was insonated with the same ultrasound probe as used for DI and spaceflight. The total energy backscattered increased by 17% compared to a plain water control "phantom". We postulate that this very basic exercise provides further evidence that calcium is reflective and a change in reflection coefficient is possible with calcium in suspension. This result need to be confirmed in a larger study.

In summary, when compared to spaceflight, DI is a model with lower fluid shift concomitant with a higher hypovolemia, a total absence of physical activity and higher stress levels due to complete immobility over several days, and lower calcium release in the blood circulation in relation to bone loss.

Conclusion

The results support the hypothesis of arterial wall remodeling and changes to wall content, observed after both a short duration space flight analog (4 days DI) and long duration (6 m) spaceflight. Moreover the coefficient of signal return as evaluated from the radio frequency (RF) signal provides information on the physical status (structure/content) of the tissues as early as after 4 days exposure to extreme environment which is not the case with other conventional ultrasound parameter like wall thickness or stiffness. This parameter will be tested on subjects in different space analog environments (ie bedrest/confinement) allowing for countermeasures like physical activity or reduced environmental stress.

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 experimental protocol conformed to the standards set by the Declaration of Helsinki and was approved by the local Ethics Committee (CPP Est III: 2 October 2018, nÂ° ID RCB 2018-A01470-55) and French Health Authorities (ANSM: 13 August 2018). Clinical Trials. gov Identifier: NCT03915457. The patients/participants provided their written informed consent to participate in this study.

Author contributions

PA designed and organized the experiment. Contribute to the data collection and analysis and wrote the original manuscript. DG made the statistical analysis and contributed to the review of the manuscript. LG contribute to the processing of the data and revised the manuscript, RH revised 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.

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Rehabilitation assisted by Space technology—A SAHC approach in immobilized patients—A case of stroke

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Introduction: The idea behind the presentation of this case relates to utilizing space technology in earth applications with mutual benefit for both patients confined to bed and astronauts. Deconditioning and the progressiveness of skeletal muscle loss in the absence of adequate gravity stimulus have been of physiological concern. A robust countermeasure to muscle disuse is still a challenge for both immobilized patients and astronauts in long duration space missions. Researchers in the space medicine field concluded that artificial gravity (AG) produced by short-radius centrifugation on a passive movement therapy device, combined with exercise, has been a robust multi-system countermeasure as it re-introduces an acceleration field and gravity load.

Methods: A short-arm human centrifuge (SAHC) alone or combined with exercise was evaluated as a novel, artificial gravity device for an effective rehabilitation strategy in the case of a stroke patient with disability. The results reveal valuable information on an individualized rehabilitation strategy against physiological deconditioning. A 73-year-old woman was suddenly unable to speak, follow directions or move her left arm and leg. She could not walk, and self-care tasks required maximal assistance. Her condition was getting worse over the years, also she was receiving conventional rehabilitation treatment. Intermittent short-arm human centrifuge individualized protocols were applied for 5 months, three times a week, 60 treatments in total.

Results: It resulted in significant improvement in her gait, decreased atrophy with less spasticity on the left body side, and ability to walk at least 100 m with a cane. Balance and muscle strength were improved significantly. Cardiovascular parameters improved responding to adaptations to aerobic exercise. Electroencephalography (EEG) showed brain reorganization/plasticity evidenced through functional connectivity alterations and activation in the cortical regions, especially of the precentral and postcentral gyrus. Stroke immobility-related disability was also improved.

Discussion: These alterations were attributed to the short-arm human centrifuge intervention. This case study provides novel evidence supporting the use of the short-arm human centrifuge as a promising therapeutic strategy in patients with restricted mobility, with application to astronauts with long-term muscle disuse in space.

KEYWORDS

artificial gravity, cardiac output, deconditioning, graph theory, mean arterial pressure, short-arm human centrifuge, rehabilitation, exercise

1 Introduction

1.1 The clinical problem

Stroke is the second most common cause of death worldwide and the most frequent cause of adult-onset and long-term acquired disability (Bonita et al., 2004; Lopez et al., 2006), leading to a harmful effect on the nervous, musculoskeletal, heart, lung, and gastrointestinal systems. Intracerebral hemorrhage (ICH) is a devastating form of stroke with a high mortality rate and poor prognosis (Mendelow et al., 2015). Nearly all the patients hospitalized with stroke suffer one or more medical or neurological adverse events (Langhorne et al., 2000; Ingeman et al., 2010). Almost 50% of deaths occurring in the first month after a stroke may be due to immobility-related adverse events (Lavados et al., 2007).

The use of gravity by means of long-term exercise combining aerobic and resistance training to enhance flexibility, balance, and coordination within daily activities for patients after a stroke has been recommended by the American Heart Association with specific guidelines published (Gordon et al., 2004). However, the restricted mobility of these patients and their inability to exercise properly lead to the need for alternative g-load rehabilitation techniques.

1.2 Strategies and evidence

Within 4 years of stroke onset, the resulting decreased physical activity (Gebruers et al., 2010) leads to fatigue within the first year (Duncan et al., 2012), and after 4 years of stroke onset, it leads to significant reductions with more than 30% evident in autonomy and in socializing participations (Gadidi et al., 2011). More than 75% of stroke patients receive rehabilitation therapy (Buntin et al., 2010), while many patients remain with residual functional disabilities, increasing the need for effective stroke care and the demand for individualized interventions (Mazrooyisebdani et al., 2018), targeted to improve stroke survivors' lives by reducing disabilities and by regaining independence (Dobkin, 2005; Bindawas and Vennu, 2016).

Space researchers recognized early on that physical inactivity and gravitational demands on the body associated with bed rest, used to simulate the physiological conditions induced by the weightlessness of spaceflight, share the same mechanisms (Pavy-Le Traon et al., 2007). If gravity is not used due to inactivity or bed rest, it leads to negative consequences in all human physiological systems (Rittweger and Frings-Meuthen, 2013; McDonnell et al., 2019) since our health depends on gravity on Earth for optimal function (Vernikos, 1996; Vernikos et al., 1996; Vernikos, 1997; Vernikos, 2008; Vernikos, 2017).

The consequences of bed rest on human physiology led Space researchers to seek effective means of counteracting the detrimental effects of the lack of gravity in space. Several countermeasures have been tested (LeBlanc et al., 1988; Norsk, 2014). More recently, the intermittent administration of Gz combined with exercise using a short-arm human centrifuge (SAHC) was proposed as the most promising countermeasure for mitigating the physiological multisystem deconditioning in space or bed rest (Vernikos, 1997; Clement et al., 2015). This evidence-based medicine was introduced in stroke patients, since research in rehabilitation after stroke revealed that interventions with physical therapy and exercise provide additive value (Dobkin, 2005).

Stroke also affects bone mineral density (BMD) and lean tissue mass (Celik et al., 2008) in both legs, but the decline is more profound in the paretic lower limb (Beaupre and Lew, 2006). This, combined with balance deficits resulting from stroke, increases the risk of falls and fractures (Eng et al., 2008). Jorgensen and Jacobsen (2001) assessed 40 patients at 6 days, 7 months, and 1 year after stroke, and they reported that the changes in BMD after stroke are correlated with functional deficits in the paretic limb, strengthening the need for an effective individualized rehabilitation strategy. Additionally, brain injury resulting from unilateral stroke alters brain function and the complex balance within the cortical activity, affecting the functional network architecture of cortical areas in both hemispheres (Van Putten and Tavy, 2004; Grefkes et al., 2011; Grefkes and Fink, 2014).

Lipnicki et al. (2009) analyzed 16 articles on studies relating to the effects of bed rest on cognitive function and suggested that exercise may improve the molecular and cellular structure and function of the brain.

Stroke patients face difficulty in moving upright in the field of gravity and exercising properly, which led to the search for methods of passively receiving the gravity load, not unlike the need for astronauts to experience gravity in space. Space technology came to the rescue with a short-arm human centrifuge, where a gravity load imposed by centripetal acceleration may be used to protect effectively various physiological systems, including skeletal muscle and bone, the cardiovascular system, and the CNS (Baker, 2007). Exercise may have additional effects on brain cortical activity in a dose–response relationship between exercise mode and intensity, affecting regions according to task familiarization and adaptation (Brümmer et al., 2011).

In the stroke patient studied here, the effectiveness of AG was evaluated by means of multiple non-invasive physiological and EEG monitoring using a series of outcome measures of the musculoskeletal system (e.g., muscle strength, mobility, and balance test), providing an estimate of changes in muscle function and sensorimotor coordination (Van Putten and Tavy, 2004), on the cardiovascular system, such as SBP, DBP, MAP, cardiac output, stroke volume, and heart rate, and the neurological system, such as functional connectivity. We anticipated alterations in functional connectivity and cortical regions' activations, especially in motor cortex regions and the regions affected by the stroke. Since physical training and aerobic exercise were associated with cortical reorganization within neuroplasticity-associated brain regions (Grefkes and Fink, 2011; Grefkes and Ward, 2014), we also anticipate enhancement of cortical excitability (Brümmer et al., 2011) due to the SAHC intervention. We would also focus on providing concrete evidence in support of "hypergravity therapy." The latter may extend the applicability of the proposed training in aging diseases, such as bone atrophy (Morita et al., 2004) and ischemia (Sang et al., 2008).

2 Materials and methods

2.1 Patient report

This case study considered a 73-year-old right-handed female patient. Her weight was 72 Kg. Her height was 150 cm. She did not face any other comorbidities apart from high blood pressure, which was well controlled with medications. She experienced, 11 years ago, an intracerebral (intraparenchymal) hemorrhage which originated within the right thalamus and extended into the right lateral ventricle with cerebral edema and midline shift. The etiology of this hemorrhage was hypertension. At the timepoint of enrollment in the study, she was alert, attentive, and oriented with clear speech and fluent with repetition, comprehension, and naming. She did not experience dysphagia or dementia. The patient had a "scissors gait" and loss of normal arm swing on the left side with clenched fist and thumb in palm (Grade 4 modified Ashworth Scale) and atrophy of the left shoulder. Her left leg was in extension with plantar flexion of the foot and toes, weakness of distal muscles, and extensor hypertonia (Grade 4 modified Ashworth Scale). She also presented pyramidal signs on her left lower extremity with un-sustained clonus and absent plantar reflex. The patient was unable to walk 3 m even with a walking aid (cane). No botulinum toxin injections for spasticity were used, and only physiotherapy was administered. Pallesthesia (tuning fork 128 Hz) and exteroceptive sensation were decreased in the left extremities compared to the right. She was treated with human centrifugation (Kourtidou-Papadeli et al., 2021; Kourtidou-Papadeli et al., 2022) for five consecutive months, three times a week for half an hour, with an intermittent gravity treatment.

2.2 Ethics

The studies involving human participants were reviewed and approved by the Bioethics Committee of the School of Medicine of the Aristotle University of Thessaloniki (179/19.03.2020). The study protocol was registered on ClinicalTrials.gov (Identifier: NCT04369976). The patients/participants provided their written informed consent to participate in this study.

2.3 Experimental design

Intermittent centrifugation was selected for rehabilitation combined with exercise since it has greater beneficial effects on the musculoskeletal and the cardiovascular system (Iwase, 2005; Clément and Bukley, 2007; Edmonds, 2008; Paloski, 2014). Additionally, with the blood flow rushing towards the feet and contracting calf muscles to increase venous return, it prevents orthostatic intolerance (OI), thus decreasing the probability of pre-syncope during acceleration (Yang et al., 2007; Yang et al., 2010; Diaz Artiles et al., 2016). Among the symptoms of OI expected were nausea, sweating, confusion, dizziness, a narrowing of the visual field, an abrupt drop in MAP of > 20 mmHg, or a critical narrowing of the pulse pressure. Termination of centrifugation was determined when presyncope occurred either subjectively when the subject was feeling symptoms like nausea, sweating, gray out, omnidirectional vertigo, head vacuum feeling, dizziness, and sudden sensation of heat or objectively by the ClearSight non-invasive medical monitor when the following findings were detected: systolic blood pressure drop-off of 15 mmHg, decline of the heart rate of 15 beats/min suddenly, or the subject showing a sustainable high tachycardia. Additionally, the subject could press the panic button in case she wanted to stop.

The experimental design consisted of three phases.

Phase 1: Familiarization

Prior to her enrollment, the patient was submitted to a 10-min centrifugation trial at 1 g for familiarization. Since she tolerated the procedure without dizziness or nausea, she was enrolled in the study. She was advised to abstain from caffeine during training days, avoid alcohol and medications during the 12 h preceding the training session, avoid eating during the preceding 2 h, and avoid heavy exercise during the preceding 24 h. She was strapped to the centrifuge of a 2-m radius with safety harnesses, placing her head stable near the center of the centrifuge to avoid the Coriolis side effect due to centrifugation.

Phase 2: Initial evaluation training

The individualized protocols were evaluated during phase 2. During the initiation of this phase, we measured the cardiovascular parameters, specifically the cardiac output (CO) and mean arterial pressure (MAP), while the patient was in a standing position. Based on these values, we plotted dose–response curves that our team constructed in our previous paper (Kourtidou-Papadeli et al., 2021). Our aim was to get an estimation of the optimal g-load training for our patient. Then, the patient was submitted to the actual centrifugation with gradually increasing gravitational loads in order to verify the determination of the optimal gravity load for the 5-month treatment based on the cardiovascular parameters. The test started at .5 g, followed by .7, 1.0, 1.2, 1.5, 1.7, and 2 g, each for 5 min, with gradual acceleration and deceleration and with 6 min of pause between gravitational loads. We selected as the starting treatment intensity, the g-load with similar cardiovascular values to standing and close to the estimated value. This was the baseline treatment intensity for the patient. The onset acceleration was 0.2 g/s. The apex of the head was in touch with the rotation axis. We used this as the reference point to measure the distance to the feet and consequently the revolutions per minute needed to generate the appropriate g-load. There was a movable board adjusted to the soles of the feet to be in close contact with the board.

Phase 3: Rehabilitation treatment

The rehabilitation approach included two different protocols: A and B. Protocol A consisted of 1.7 g (6 min), 4 min break/1.7 g (6 min), 4 min break/1.7 g (6 min), 4 min break/1.7 g (6 min), 4 min break/2 g (6 min), and protocol B consisted of 2 g (10 min), 4 min break, 2.2 g (10 min), 4 min break, 2.5 g (10 min) with SAHC alone and the same protocols combined with exercise, resulting in five conditions as pre-SAHC, protocol A, protocol A + exercise, protocol B, and protocol B + exercise of 5 weeks. Each condition included 15 sessions of centrifugation, with measurements of the strength and balance taken after 5 min standing for "pre" and 5 min after the end of centrifugation for "post." The EEG data were taken before entering the rehabilitation with SAHC and after each protocol. The total rehabilitation treatment was 5 months. Each measurement of grip strength, posturography, and balance was performed three times, and the best effort was accepted. The protocol' description is presented in Table 1.

TABLE 1 Different protocols applied on SAHC.

Protocols SAHC	Centrifugation loads and exercise
Protocol A	1.7 g (6 min), 4 min break/1.7 g (6 min), 4 min break/1.7 g (6 min), 4 min break/1.7g (6 min), 4 min break/2 g (6 min)
Protocol A + exercise	1.7 g (6 min), 4 min break/1.7 g (6 min), 4 min break/1.7 g (6 min), 4 min break/1.7 g (6 min), 4 min break/2 g (6 min) + bicycle + arm/upper limbs resistance
Protocol B	2 g (10 min), 4 min break, 2.2 g (10 min), 4 min break, 2.5 g (10 min)
Protocol B + exercise	2 g (10 min), 4 min break, 2.2 g (10 min), 4 min break, 2.5 g (10 min) + bicycle + arm/upper limbs resistance

Each training session consisted of a total of 30 min intermittent centrifugation with 4 min pause in between. The total time of the session (including preparatory and recovery steps) was approximately 1 h and a half, and the training frequency was three times per week.

Each protocol lasted 5 weeks with a total rehabilitation time of 5 months. During centrifugations, cardiovascular and electroencephalographic (EEG) responses were continuously monitored. Before and after the 5-month rehabilitation program, neurological and physical performance tests were also carried out. Additionally, before each centrifugation session, balance and muscle strength were assessed.

During rotation, the participant's eyes were covered with an eye cover, and lights were off to remove visual cues. Then, she was instructed to avoid moving her head to avoid the Coriolis effect.

In order to safely use the centrifuge, the participant was required to fulfill some requirements. She was healthy, with a height not exceeding 2 m, and with impaired mobility from a stroke. She did not have any neurological or psychiatric disorder, vertigo, nausea or chronic pain, chronic use of substances or alcoholism, recent (within 6 months) surgery, current arrhythmia, severe migraines, pregnancy, epilepsy, chololithiasis or kidney stones, dehydration, recent wounds from surgery, recent fractures (unless recommended by a doctor), acute inflammation or pain, and newly inserted metal pins or plates or newly implanted stents.

2.4 The human centrifuge

2.4.1 Theoretical concept

SAHC is an integrated multi-system countermeasure in order to provide artificial gravity (AG) loads for rehabilitation purposes in case of physiological deconditioning due to inactivity or lack of gravitational load in the Gz direction. The AG functions by exerting a centrifugal force on a body, accelerated centripetally in a rotating device (Vernikos, 1996; Yang et al., 2010). The participant lies in a supine and horizontal position on the rotation bed, with the head towards the center. The patient is forced away from the axis of rotation with a force that is the product of body mass, distance from the axis of rotation, and angular velocity squared. This force can be calculated from the equation $F = mrw^2$, where m is the mass of the subject, r is the distance from the center of the centrifuge to the center of mass of the subject, and w is the velocity at which the bed rotates. Thus, the force exerted at the feet increases with the velocity of rotation and with the body's distance from the axis of rotation. The reference point for the g-level applied is the feet of the subject. The head of the participant is aligned 10 cm close to the center of centrifuge rotation. So, when regarding a subject of 1.70 m height (+10 cm of the distance to the top of the head = 1.80 m) with a gravity gradient of 1.0 g (23 rotations/minute) at the feet, the g-load on top of the head is .06. This design greatly minimizes a Coriolis side effect, while the g-load on the feet may reach 3.5 *g* at the maximal velocity of the device. Moreover, the AG level plays a role in the magnitude of the Coriolis effect, as well as the patient's head movement, since Coriolis force also derives from the mismatch of vestibular information and the effect of Earth's gravity.

2.4.2 The short-arm human centrifuge architecture

The short-arm human centrifuge (SAHC) was developed by our research group (patent #1009812/13/09/2019) and is located at the "Joan Vernikos" Laboratory of Aerospace and Rehabilitation Applications. The diameter is 4 m, with the possibility of reaching a gravity load at the feet of +3.5 Gz. It consists of a support base: a central axis with two beds connected and rotating smoothly on wheels. Our previous experiments validated the mechanical aspects of the centrifuge and the successful attainment of physiological data during centrifugation and exercise. Subjects with neurodegenerative diseases well tolerated the centrifugation and successfully completed the exercise protocol.

2.4.3 Aerobic exercise intervention

The centrifugation on the SAHC was combined with mild-intensity exercise based on both percentage of predicted maximal heart rate (%PMHR) and the Karvonen formula (Karvonen et al., 1957). Mild-intensity exercise includes 40%–59% maximal heart rate (MHR) and 30%–49% Karvonen, corresponding to 5–8 metabolic equivalents (METS) (Powers and Howley, 1997, p.292; Wilmore and Costill, 1994, p.524, deVries and Housh, 1994, p.297). Karvonen formula was a reasonably accurate method for estimating exercise intensity, as demonstrated by Davis and Convertino (1975). Both methods seemed to be similar. The subjects were monitored during centrifugation using a Bluetooth Polar H10 device with an accurate heart rate sensor, and the intensity of the exercise was kept at "light intensity". The subject was continuously monitored and advised accordingly to ensure the exercise pace remained within the predetermined limits.

2.4.4 Aerobic exercise infrastructure

The artificial gravity training is further enhanced through a state-of-the-art aerobic exercise infrastructure. It consists of a bicycle ergometer, feet resistance, and arm/upper limb elastic band resistance.

Removable exercise equipment was attached to the bed extremities with the possibility of aerobic training through a bicycle ergometer adjusted at the feet according to the patient's height and resistance training through a horizontal rowing device for the lower limbs with elastic bands attached to the axis of the device for the arm/upper limbs.

It has been constructed and evaluated in healthy individuals according to both national and international safety regulations, while also being applicable to patients with mobility disabilities.

2.5 Neurological/medical description

The Berg Balance Scale (BBS) was used for estimating the fall risk of the patient (Blum and Korner-Bitensky, 2008). This balance score is associated with quality of life (Schmid et al., 2013) and is a sensitive (80%) and specific (78%) tool for identifying individuals at risk of falling following a stroke (Maeda et al., 2009).

2.6 Electroencephalographic analysis

2.6.1 Data acquisition

The data acquisition was performed in three experimental time instances: before SAHC, SAHC alone in both protocols, and SAHC combined with exercise on the resting state (eyes closed) condition. The patient was instructed to close her eyes and remain calm for approximately 5 min. In case there were artifacts due to body movements, opening of eyes, or a muscle/body movement, the recording session was extended proportionally. The room temperature was kept at a relatively stable condition (22°C) through artificial cooling, and the room lights were turned off. The operators also kept auditory noise minimal.

The EEG device was the 32-channel Neurofax EEG-1200 connected to a laptop (Nihon Kohden, Tokyo, Japan). Among those electrodes, there were 1) 19 EEG electrodes placed according to the 10–20 International System, 2) two electromyographic/EMG electrodes for recording chin movements, 3) two electrodes for electroculogrammic/EOG (both horizontal and vertical) recordings, and finally, 4) two electrocardiographic/ECG electrodes. Apart from the electrodes used for EEG data, all the others (ECG, EMG, and EOG) were bipolar. The ground electrode was placed on the prefrontal midline (Fpz) position. There were also two reference electrodes placed on the left and right mastoid muscles. The sampling frequency was kept at 500 Hz for all the signals.

2.6.2 EEG Preprocessing

This step was performed through custom scripts on Python 3.6. First, we performed a common average re-referencing. Then, we applied several Butterworth filters of second order with the following order:

- High-pass filter with the cut-off frequency at .5 Hz
- Low-pass filter with the cut-off frequency at 50 Hz
- Three band-stop (notch) filters centered at 50, 100, and 150 Hz, respectively

The high-pass filter was used to remove DC shifts (linear trends). The low-pass filter was used for removing high-frequency noise. The first band-stop filter was used for removing the industrial noise induced by the power supply, and the other ones were for removing the industrial noise harmonics. Then, the filtered data were subjected to independent component analysis as implemented through the EEGLAB graphical user interface under the MATLAB environment. Two experienced neuroscientists inspected the data in order to recognize and remove artifactual sources. Then, visual inspection was also performed on the electrodes' time series to remove time segments that were still heavily contaminated with noise. The remaining data were divided into non-overlapping epochs. The epoch duration was 16 s.

2.6.3 Cortical functional connectivity analysis

The analysis was performed through the Brainstorm graphical user interface and custom scripts in MATLAB. The Brainstorm was used for modeling the generic head anatomy by employing the M/EEG boundary element method. We employed the default Brainstorm settings for modeling the cortex by means of 15,000 dipoles of fixed orientation. The estimation of the cortical activity was performed by solving the inverse problem through the sLORETA methodology as implemented within the Brainstorm software. Then, we grouped the 15,000 dipoles into 148 cortical regions according to the Destrieux atlas (Destrieux et al., 2010). This is a probabilistic sulco–gyral atlas of the human cerebral cortex. The cortical activity of each region was estimated as the mean value of all the dipoles consisting of that region. Then, we performed functional connectivity analysis through the synchronization likelihood/SL method (Stam and Van Dijk, 2002). This method was selected since it is superior to linear and symmetric (coherency) metrics by avoiding the bias of the freedom degrees and its robustness when dealing with non-stationary dynamics (Montez et al., 2006). Its output is a 148 × 148 connectivity matrix. Each cell of this matrix denotes the co-operative degree among the given pair of cortical regions. The values range from 0. . .1. Lower values denote minimal connectivity among the specific pair, whereas larger values denote stronger co-operative activity. The main diagonal line is equal to 1, since each region is compared with itself.

2.6.4 Cortical network analysis

The analysis was performed through custom MATLAB scripts, which employed functions derived from the Brain Connectivity Toolbox (BCT). We used binary, non-directed graphs by detecting the 20% largest pairs of connectivity values. This was achieved by applying an adaptive threshold for each epoch to ensure that all the graphs were of the same density. The selected edges were binarized to "1", while all the other pairs of the connectivity matrix were set as "0". Then, we estimated global network metrics such as the mean cluster coefficient, the characteristic path length, the global efficiency score, and the modularity score. Since we were interested in the identification of the most prominent hubs, we also estimated the normalized betweenness centrality metric.

Then, we computed the following network metrics:

- Node degree: It is the number of edges that each node contains.
- Distance matrix: It is computed as the shortest path among two nodes. Therefore, it is the minimum number of edges needed to reach from one node to any other.
- Characteristic path length: It is the mean value of the shortest paths among all node pairs.

2.7 Cardiovascular analysis

2.7.1 Cardiovascular measures

Systolic blood pressure (SBP) mmHg, diastolic blood pressure (DBP) mmHg, mean arterial pressure (MAP) mmHg, cardiac output (CO) L/min, stroke volume (SV) mmHg, and heart rate (HR) were monitored continuously and non-invasively with finger plethysmography with the ClearSight device (Edwards Life Sciences) (Figure 1).

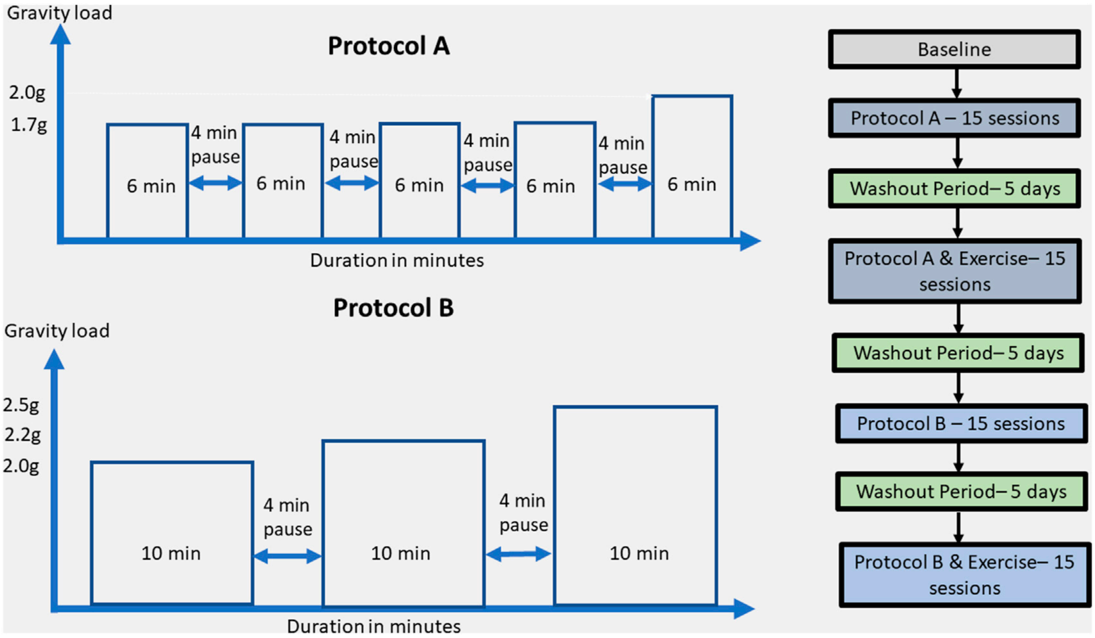

FIGURE 1
Detailed description of the protocol A and B training components on the upper left and right side, respectively. The entire rehabilitation approach in terms of protocols, exercise presence or not, wash-out periods, and number of sessions is described in the right part of the figure.

FIGURE 2
Finger plethysmography (left side) instant values of cardiovascular parameters monitored by the ClearSight device connected to the patient during centrifugation and recording data every 30 s. SAHC with the patients on the beds, one at the left bed with bicycle ergometer and the other with SAHC only and simultaneous recordings is visualized on the right side of the screen.

2.8 Muscle analysis

2.8.1 Grip strength

The outcome measures derived from this device are max. strength % (MS%), max. strength in kilograms (MSKg), and max. strength deficit % (MSD%). Grip strength has been found to be a useful objective measure of motor impairment, particularly the rate of increase in grip forces (Renner and Bungert-Kahl, 2009). To evaluate, reassess, and monitor grip strength, the K-FORCE grip dynamometer developed by KINVENT company was used, which works *via* Bluetooth Android application (Figure 2).

TABLE 2 Descriptive statistics for the right postcentral gyrus, where "pre" stands for before SAHC, "mid" stands for SAHC alone in both protocols, and "post" stands for SAHC combined with exercise.

Network metric	Mean value ± standard deviation			Minimum value			Maximum value		
	Training phase			Training phase			Training phase		
	Pre	Mid	Post	Pre	Mid	Post	Pre	Mid	Post
Cluster	.636 ± .063	.438 ± .074	.478 ± .074	.504	.318	.364	.775	.632	.683
Betweenness centrality	.420 ± .237	1.265 ± .891	1.880 ± .956	.085	.399	.615	.983	3.105	3.644
Node degree	70.889 ± 13.164	65.333 ± 18.111	90.111 ± 17.129	50	38	68	98	104	118

2.8.2 Posturography and balance

Static and dynamic posturography, weight distribution difference (WDD), average position of the center of pressure (APCOP) in the medio-lateral and antero-posterior planes, and average velocity of displacement (AV) were evaluated with a frequency of up to 75 Hz. These data were processed with KINVENT's Balance Clinic software (Figure 2).

2.9 Statistical analysis

For Statistical analysis, Python 3.8.5 and R Studio 1.3.1093 were used on a Windows PC. The level of statistical significance was set to $p < .05$. For continuous variables, we calculated descriptive statistics (through tables and boxplots), means, and standard deviations, and for categorical variables, absolute and relative frequencies were calculated. One-way, repeated measures analyses of variance (ANOVAs) were conducted for each independent categorical variable. When sphericity was not assumed, Greenhouse–Geisser corrections were applied.

3 Results

3.1 Cortical network characteristics

The measurements were performed in three-time experimental instances: before SAHC (Pre), SAHC alone in both protocols (mid), and SAHC combined with exercise (post). The analysis was focused on the right side of the brain, which affected the left side of the patient.

3.1.1 Right postcentral gyrus

The descriptive statistics of the network characteristics for the right postcentral gyrus in the various time instances are reported in Table 2:

There was a statistically significant main effect of the intervention on the 1) cluster coefficient $F(1,34) = 64.953$, $p < .0001$, 2) betweenness centrality metric $F(1,34) = 17.975$, $p < .0001$, and 3) node degree $F(1,34) = 11.391$, $p = .0002$.

Post hoc comparisons performed regarding the Cluster coefficient identified a statistically significant difference between pre and mid values ($t = 10.708$, $p < .001$) and pre and post values ($t = 13.360$, $p < .001$) and a marginal significance between mid and post values ($t = -1.746$, $p = .099$).

Post hoc comparisons performed regarding the betweenness centrality metric identified a statistically significant difference between pre and mid values ($t = -4.071$, $p = .001$) and pre and post values ($t = -7.320$, $p < .001$) and a marginal significance between mid and post values ($t = -1.98$, $p = .064$).

Post hoc comparisons performed regarding the node degree did not identify a statistically significant difference between pre and mid values ($t = .964$, $p = .349$). However, there was a statistically significant difference between pre and post values ($t = -5.149$, $p < .001$) and between mid and post values ($t = -3.830$, $p = .001$).

3.1.2 Right precentral gyrus

The descriptive statistics of the network characteristics for the right precentral gyrus in the various training phases are reported in Table 3:

There was a statistically significant main effect of the intervention on the 1) cluster coefficient $F(1,34) = 6.976$, $p = .0029$, 2) betweenness centrality metric $F(1,34) = 6.88$, $p = .003$, and 3) node degree $F(1,34) = 13.529$, $p < .0001$.

Post hoc comparisons performed regarding the cluster coefficient identified a statistically significant difference between pre and mid values ($t = 4.085$, $p = .001$), no statistical significance between pre and post values ($t = 1.460$, $p = .163$), and a marginal significance between mid and post values ($t = -2.081$, $p = .053$).

Post hoc comparisons performed regarding the betweenness centrality metric did not show a statistically significant difference between pre and mid values ($t = -1.640$, $p = .119$). There were statistically significant differences between pre and post values ($t = -3.167$, $p = .006$) and between mid and post values ($t = -2.286$, $p = .035$).

Post hoc comparisons performed regarding the node degree did not show a statistically significant difference between pre and mid values ($t = .778$, $p = .447$). There were statistically significant differences between pre and post values ($t = -4.471$, $p < .001$) and between mid and post values ($t = -4.477$, $p < .001$).

The differences for both cortical regions are visualized in Figure 3.

3.2 Muscle analysis results

Table 4 visualizes the statistically significant main effects for the various independent variables regarding the muscle analysis. The muscles that are involved are the grip muscles, and among them are the flexor digitorum profundus, flexor digitorum superficialis, flexor digiti minimi brevis, flexor pollicis longus, extensor digitorum,

TABLE 3 Descriptive statistics for the right precentral gyrus, where "pre" stands for before SAHC, "mid" stands for SAHC alone in both protocols, and "post" stands for SAHC combined with exercise.

Network metric	Mean value ± standard deviation			Minimum value			Maximum value		
	Training phase			Training phase			Training phase		
	Pre	Mid	Post	Pre	Mid	Post	Pre	Mid	Post
Cluster	.596 ± .077	.503 ± .082	.559 ± .079	.439	.385	.392	.772	.693	.708
Betweenness centrality	.633 ± .436	.870 ± .499	1.344 ± .724	.100	.218	.488	1.803	1.988	3.002
Node degree	72.222 ± 14.996	67.667 ± 18.436	96 ± 17.026	46	38	64	100	102	138

FIGURE 3
Max. grip strength dynamometer assessment (patient at the right lower corner) with the results at the left upper corner and the improvement of the studying period. Posturography K-FORCE plates, as seen at the left lower corner, for posture and stance evaluation, center of pressure, and weight distribution measurement.

lumbricals, interossei, and adductor pollicis. The measurements performed were the 1) maximum strength percentage, 2) maximum strength, 3) weight distribution percentage, 4) total range, 5) maximum strength deficit (Percentage), 6) total CoP displacement, and 7) average velocity. The maximum strength percentage, maximum strength percentage, and weight distribution percentage were analyzed on the left and the right body sides, and the total range was analyzed on lateral and longitudinal positions, while the remaining ones (maximum strength deficit, total CoP displacement, average velocity) were analyzed. Then, we performed Tukey *post hoc* comparisons for the independent variables that induced either a main effect or an interaction effect.

Tukey *post hoc* comparisons for the independent variables induced either a main effect or an interaction effect. The results are reported in the following sub-sections and visualized in Figures 4, 5.

3.2.1 Maximum strength percentage

When considering the left side of the body, there was a statistically significant difference between 1) A and B protocols when there is no exercise ($p = .036$) (Figure 4), 2) regarding the A protocol between no exercise and exercise existence ($p = .0069$) (Figure 4), 3) protocol A with exercise vs. protocol B with no exercise ($p < .0001$) (Figure 4), 4) protocol B with and without exercise ($p = .003$) (Figure 4), and 5) marginally statistically significant differences regarding protocol A and protocol B with exercise ($p = .08$).

TABLE 4 Visualization of the main effects of protocol, exercise, and their interaction on the various muscle variables.

Independent variable	Protocol		Exercise		Protocol × exercise	
	Left	Right	Left	Right	Left	Right
Maximum strength percentage	F (1,56) = 13.795, p = .0005	F (1,56) = 17.245, p = .0001	F (1,56) = 25.124, p < .0001	F (1,56) = 29.176, p < .0001		
Maximum strength		F (1,56) = 25.138, p = <.0001	F (1,56) = 23.267, p = <.0001			F (1,56) = 11.800, p = <.0011
Weight distribution percentage			F (1,56) = 4.66, p = <.0352			
	Lateral	Longitudinal	Lateral	Longitudinal	Lateral	Longitudinal
Total range				F (1,56) = 8.156, p = <.006		
Maximum strength deficit (percentage)	F (1,56) = 14.120, p = <.0004		F (1,56) = 24.562, p = <.0001			
Total CoP displacement						
Average velocity			F (1,56) = 6.192, p = <.0158			

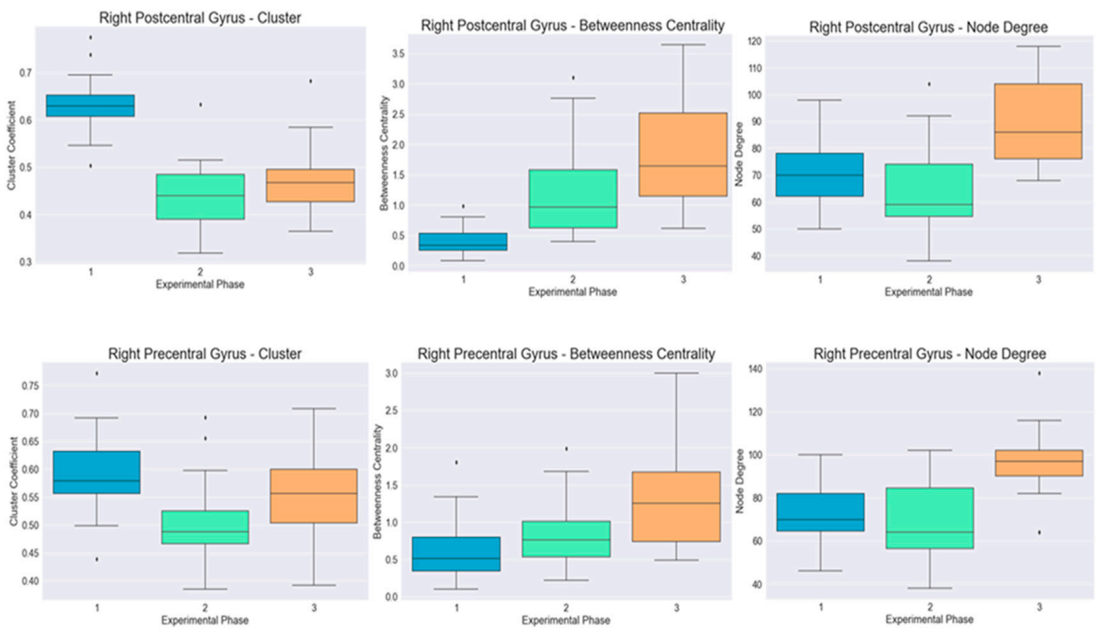

FIGURE 4
Visualization of the distribution of the cluster coefficient, betweenness centrality, and node degree for the right postcentral and right precentral gyrus across the three-time instances during the experimental phase, where "1" stands for before SAHC, "2" stands for SAHC alone in both protocols, and "3" stands for SAHC combined with exercise. For clarification, both AG alone or if combined with exercise is considered treatment. So, the only "pre" period is before centrifugation, and every protocol after that stands for "post."

When considering the right side of the body, there was a statistically significant difference between 1) A and B protocols when there is no exercise (p = .047) (Figure 4), 2) regarding the A protocol between no exercise and exercise existence (p = .0008) (Figure 4), 3) protocol A with exercise vs. protocol B with no exercise (p < .0001) (Figure 4), 4) protocol B with and without exercise (p = .004), and 5) protocol A and protocol B with exercise (p = .012).

3.2.2 Maximum strength

When considering the left side of the body, a statistically significant difference regarding 1) the A protocol between no exercise and exercise existence (p = .0003) (Figure 5), 2) protocol A with exercise vs. protocol B with no exercise (p = .0002) (Figure 5), and 3) marginal statistically significant difference regarding the protocol B with and without exercise (p = .082) (Figure 5).

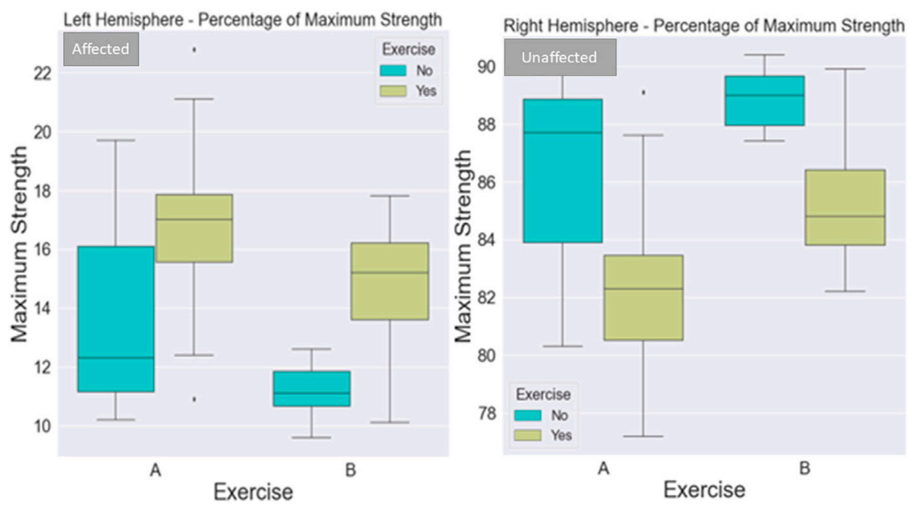

FIGURE 5
Visualization of maximum strength percentage for the left and right sides of the body. The maximum strength of the affected side (left part of the image) improved with exercise in both protocols. The maximum strength of the non-affected side (right part of the image) improved with both protocols when not combined with exercise. The exercise added to the centrifugation was very light to make a difference for the unaffected side.

When considering the right side of the body, there was a statistically significant difference between 1) A and B protocols when there is no exercise ($p < .0001$) (Figure 5), 2) marginal statistically significant difference regarding the A protocol between no exercise and exercise existence ($p = .077$), 3) protocol B with exercise vs. protocol A with no exercise ($p = .004$) (Figure 5), 4) protocol A with exercise vs. protocol B with no exercise ($p = .005$) (Figure 5), and 5) marginal statistically significant difference regarding the protocol B with and without exercise ($p = .09$).

3.2.3 Weight distribution percentage

When considering the left side of the body, there was a marginally statistically significant difference regarding protocol A, with versus without exercise ($p = .086$). Since there were no statistically significant differences in the right side of the body, no *post hoc* comparisons were performed.

3.2.4 Total range

Since there were no statistically significant differences regarding the lateral parameter, no *post hoc* comparisons were performed.

When considering the longitudinal parameter, there was a statistically significant difference regarding protocol A, with versus without exercise ($p = .040$), and protocol A with versus protocol B without exercise ($p = .024$).

3.2.5 Maximum strength deficit (percentage)

There was a marginal statistically significant difference between protocols A and B without exercise ($p = .055$). There was a statistically significant difference regarding protocol A with versus without exercise ($p = .004$). There was a statistically significant difference between protocol A with exercise versus protocol B without exercise ($p < .001$). There was a statistically significant difference regarding protocol B with versus without exercise ($p = .006$). There was a marginal statistically significant difference between protocols A and B with exercise ($p = .043$).

3.2.6 Total CoP displacement

Since there were no statistically significant differences for this feature, no *post hoc* comparisons performed.

3.2.7 Average velocity

There was a statistically significant difference regarding protocol B with versus without exercise ($p = .049$).

3.3 Cardiovascular analysis results

Since our analysis aims to investigate the impact of two independent variables (both protocol and exercise type) on several cardiac markers, we wished to detect either a significant main effect of each variable or an interactive one. Therefore, we chose to visualize the distribution of the cardiac markers (A: cardiac output, B: cardiac index, C; stroke volume, D: pulse rate, E: mean arterial pressure, and F: systolic pressure) during the various exercise conditions through two different graphs (Figure 6).

Figure 7 represents the variation of the cardiac markers according to the different protocols (1: protocol A, 2: protocol B), while Figure 8 represents the impact of exercise (1: no exercise; 2: exercise existence). So, Figure 7 merges the exercise type and compares protocol A with or without aerobic exercise versus protocol B with or without exercise. Both Figures 7, 8 contain six violin plots, each one corresponding to the six SAHC g-loads (1: lying, 2: 1.5 g, 3: 1.7 g, 4: 1.7 g, 5: 1.7 g, and 6: 1.7 g) which are part of the proposed intervention procedure. On the other hand, we compare both protocols without exercise versus both protocols with exercise in Figure 8. Although boxplots are much more frequent in biomedical research studies, we selected violin plots since they are much more informative. Box plots contain the summary statistics, whereas violin plots visualize the entire data distribution. So, it is of particular importance to understand the peak distribution and the existence of potential outliers.

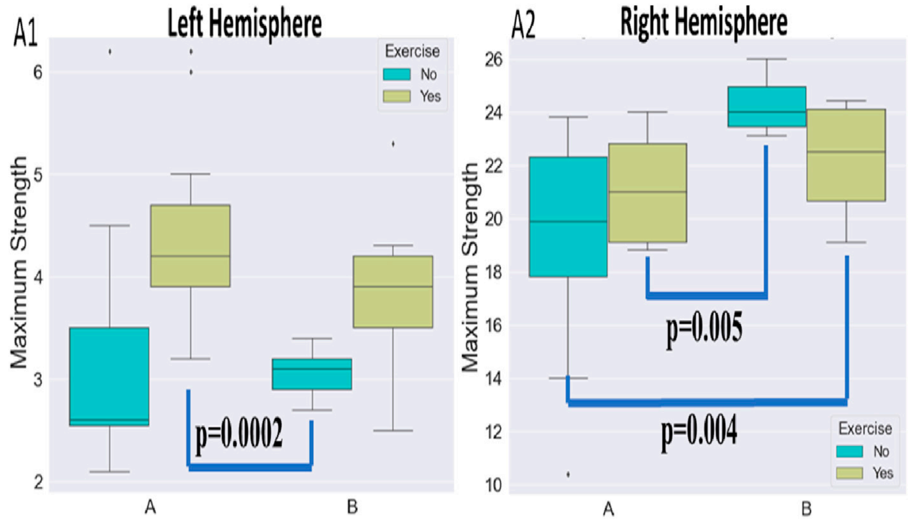

FIGURE 6
Visualization of maximum strength alterations for the left **(A1)** and right **(A2)** hemisphere and the various protocols.

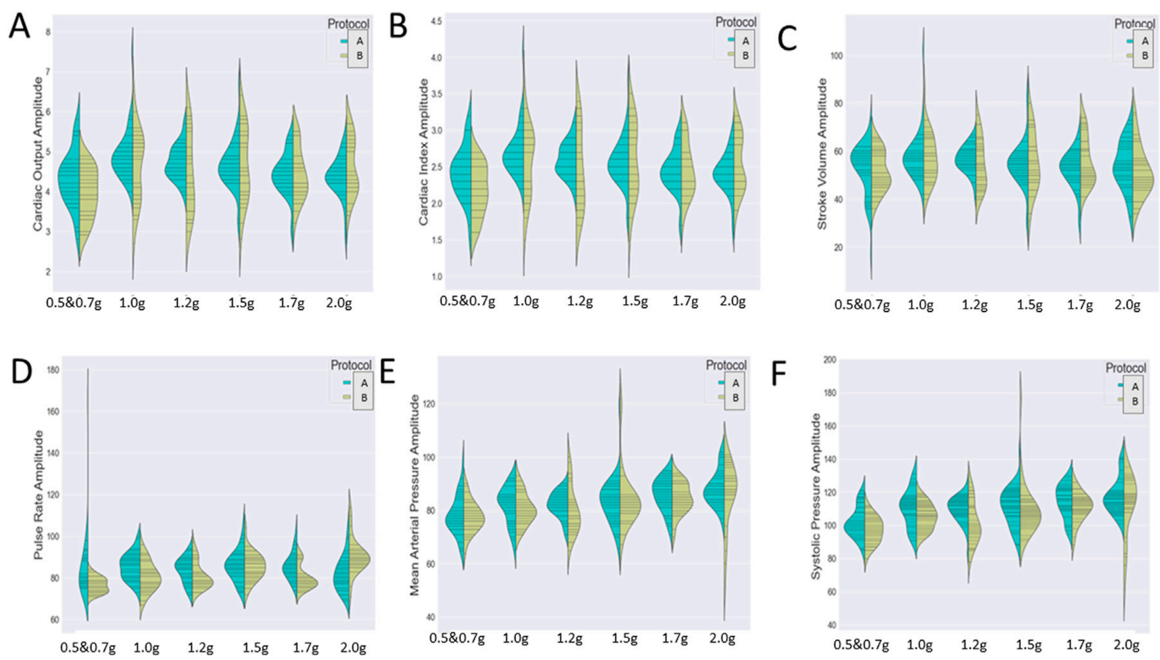

FIGURE 7
Visualization of six cardiac markers [**(A)** cardiac output, **(B)** cardiac index, **(C)** stroke volume, **(D)** pulse rate, **(E)** mean arterial pressure, and **(F)** systolic pressure] comparing the effect of protocols A (1) and B (2) either combined with exercise or not. The number of measurements for SAHC alone (both protocols) was 30 measurements (three measurements per week), and that for combined with exercise (both protocols) was also 30 measurements. For the grip strength and balance, the measurements were taken upon arrival after 5 min standing for the "pre" values and 5 min after the end of centrifugation for the "post" values. The EEG data were taken before entering the rehabilitation with SAHC and after each protocol. The figure visualizes the distribution of each marker's instance for AG training protocol A alone and combined with exercise (green color) and protocol B alone and combined with exercise (yellow color). Elongated violin plot edges represent the existence of outliners, while their width is proportional to the existence of the instances.

There was a statistically significant *protocol* × *exercise* × *condition* triple interaction for *pulse rate*: [F (1,388) = 4.055, p = .04473].

There was a statistically significant *protocol* × *condition* double interaction for 1) *cardiac output*: [F (1,388) = 8.827, p = .0032], 2) *cardiac index*: [F (1,388) = 10.294, p = .00145], and 3) *pulse rate*: [F (1,388) = 24.699, p < .0001].

There was a statistically significant *exercise* × *condition* double interaction for 1) *stroke volume*: [F (1,388) = 5.055, p = .0251], 2) *stroke*

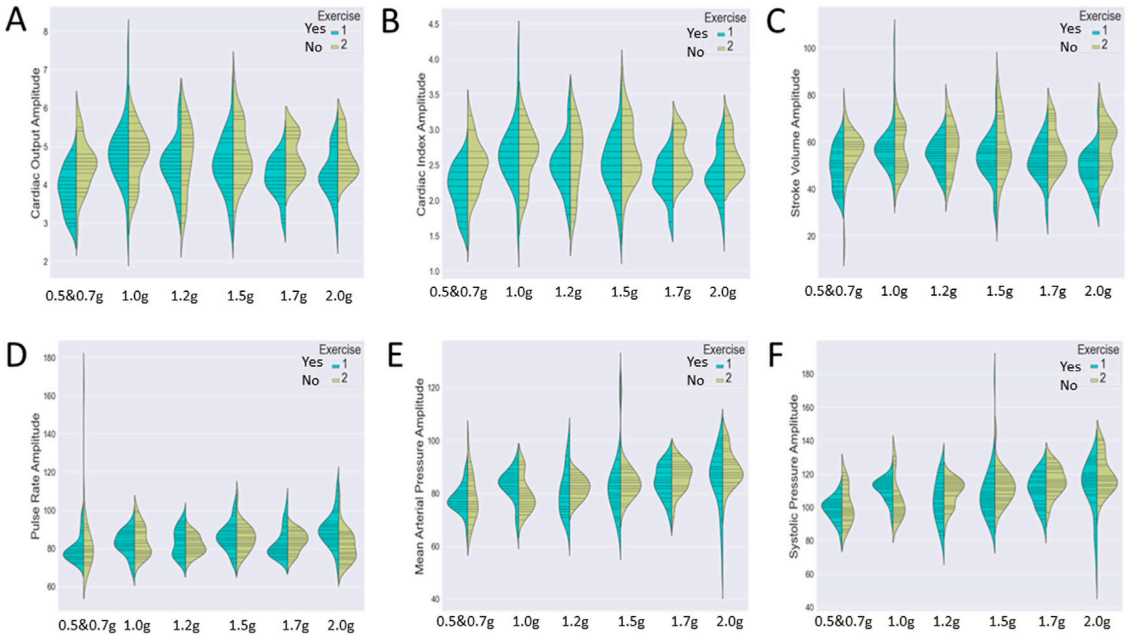

FIGURE 8
Visualization of six cardiac markers [**(A)** cardiac output, **(B)** cardiac index, **(C)** stroke volume, **(D)** pulse rate, **(E)** mean arterial pressure, and **(F)** systolic pressure] comparing the effect of exercise, independently of the protocol. The number of measurements for SAHC alone (both protocols) was 30 measurements (three measurements per week), and that for combined with exercise (both protocols) was also 30 measurements. For the grip strength and balance, the measurements were taken upon arrival after 5 min standing for the "pre" values and 5 min after the end of centrifugation for the "post" values. The figure visualizes the distribution of each marker's instance for AG training protocols A and B together with no exercise (1) (green color) and protocols A and B together with exercise (2) (yellow color) through ergometer and resistive exercise of arm/upper limb. Elongated violin plot edges represent the existence of outliners, while their width is proportional to the existence of the instances.

volume index [F (1,388) = 5.277, p = .0221], 3) *pulse rate* [F (1,388) = 8.947, p < .00296], 4) *systolic pressure*: [F (1,388) = 8.518, p = .004], and 5) *mean arterial pressure*: [F (1,388) = 4.430, p = .0359].

There was a statistically significant *protocol* × *exercise* double interaction for 1) *systolic pressure:* [F (1,388) = 4.971, p = .02635] and 2) *mean arterial pressure* [F (1,388) = 4.685, p = .0310].

There was a statistically significant main effect of exercise for 1) *cardiac output*: [F (1,388) = 24.803, p < .0001], 2) *cardiac index*: [F (1,388) = 22.830, p < .0001], 3) *stroke volume*: [F (1,388) = 27.460, p < .0001], 4) *stroke volume index*: [F (1,388) = 27.825, p < .0001], and 5) *systolic pressure*: [F (1,388) = 5.732, p = .0171].

There was a statistically significant main effect of protocol for 1) *pulse rate*: [F (1,388) = 7.286, p = .00725] and 2) *systolic pressure* [F (1,388) = 17.728, p < .0001].

There was a statistically significant main effect of condition for 1) *pulse rate*: [F (1,388) = 9.629, p = .00206], 2) *systolic pressure*: [F (1,388) = 71.306, p < .0001], 3) *diastolic pressure*: [F (1,388) = 29.845, p < .0001], and 4) *mean arterial pressure*: [F (1,388) = 58.771, p < .0001].

3.4 Neurological evaluation results

The Berg Balance Scale (BBS) score of the patient, when enrolled in the study, was = 6, which is interpreted as almost 100% fall risk and the patient is either already wheelchair-bound or may be soon. The improvement of the BBS after the treatment was 26, while the patient now may require assistance in performing certain activities of daily living, such as walking.

Somatosensory impairments: the patient presented somatosensory impairments of both left upper and lower limbs, with no sensory reaction before rehabilitation and started feeling the paretic side in the first month of rehabilitation; she could feel and move the upper and lower limb in the second month, could stand with no cane in the third month, and could walk several steps without a cane at the end of the treatment period of 5 months. After centrifugation, the gait was much better with less spasticity (no scissors gait) on the left body side. There was still no arm swing, and the spasticity of the left fist was better with no thumb in palm. There was a decrease in left shoulder atrophy. Pyramidal signs of the left lower extremity remained (unsustained clonus and absent plantar reflex). The patient, after a 5-month treatment period, was able to walk 100 m with a cane.

4 Discussion

The main finding of this study was the effectiveness of the SAHC alone or combined with aerobic and resistive exercise on all cardiovascular parameters, on strength and functional activities of both paretic and normal upper limb, as well as posturography and balance, leading to a significant decrease in disability. When the centrifuge was acting alone, then the protocol played a significant role. When protocols were combined with exercise, both were effective. The EEG graph theory analysis revealed neuroplasticity

TABLE 5 Comparison of protocols for the grip variables.

Variables	Protocol a	Protocol B	+Exercise	Hemiparietic L. side (mean ± sd)	Normal R. side (mean ± sd)
Grip max. strength% (MS%)	✓	—	—	13.7 ± 3.22	86.3 ± 3.22
	✓	—	✓	16.8 ± 2.94	82.5 ± 3.24
	—	✓	—	11.1 ± .866	88.9 ± .977
	—	✓	✓	14.5 ± 2.31	85.5 ± 2.31
Max. strength (MSKg)	✓	—	—	3.11 ± 1.13	19.0 ± 3.75
	✓	—	✓	4.4 ± .826	21.2 ± 1.93
	—	✓	—	3.06 ± .22	24.2 ± .973
	—	✓	✓	3.77 ± .756	22.1 ± 1.95
Maximum strength deficit percentage (MSD%)	✓	—	—	84.0 ± 4.44	
	✓	—	✓	79.4 ± 4.52	
	—	✓	—	87.4 ± 1.17	
	—	✓	✓	82.9 ± 3.10	

For the normal side (R), the grip max. strength% (MS%) and the max. strength (MSKg) of protocol B was more effective than that of protocol A (dark color). For the paretic side (L), protocol A combined with exercise was more effective (dark color). Less max. strength deficit appeared with protocol A combined with exercise. The tick stands for statistically significant improvement, and the negative sign stands for no statistically significant improvement.

with significant improvement after treatment in the areas of the right precentral gyrus and right postcentral gyrus areas.

Hemiplegia and, consequently, the deterioration of motor skills play a major role in the reduction of the activities of daily living and socialization (Dijkerman et al., 2004). It is well-documented that upper and lower extremity weakness in adults with stroke is related to functional disabilities (Pohl et al. 2000), (Kim et al., 2005). In total, about 85 percent of stroke patients show upper limb disorders in the acute stage (Ryerson, 2001), which increases along the way to 55%–75% after 3–6 months post-stroke (Olsen TS, 1990). A stroke involving the anterior cerebral artery (ACA) will affect the precentral gyrus, presenting with contralateral leg weakness with upper motor signs (Schneider and Gauiter, 1994). The movement limitations from the permanently affected upper limb for performing activities of daily living (Akbari et al., 2011) do not make it easy for post-stroke patients to live normal lives (Kim et al., 2014), emphasizing the importance of improvement of the upper limb functions in the rehabilitation treatment regimens (Paik and Kim, 2010).

Grip strength has been suggested as an important variable to measure after strokes. Boissy et al. (1999) investigated grip strength in 15 persons with chronic stroke and demonstrated that the strength in the more affected hand was significantly associated with the degree of disability of the upper extremity. They also showed that persons with equal grip strength in the more affected hand had almost normal upper extremity function. Moreover, in longitudinal studies, grip strength has been shown to predict motor function in the upper extremity from a short-term and long-term perspective (Heller et al., 1987; Sunderland et al., 1989).

Our findings on grip strength after centrifugation showed a significant improvement in both the normal and paretic sides, but regarding the normal side (R), the grip max. strength% (MS%) and the max. strength (MSKg) had statistically significant improvement with protocol B (a more intense protocol) compared to protocol A, with no significant differences when exercise was added to the centrifugation (Tables 4, 5). This might be due to mild-intensity exercise. But for the paretic side (L), protocol A combined with exercise was more effective for grip strength with less max. strength deficit. Grip exercise by the non-paretic hand was found to be effective in increasing the venous flow volume in the paretic hand, in accordance with the literature (Hayashi and Abe, 2020).

The decrease of muscle force in the paretic upper limb may be due to neural factors that affect motor control and also due to inactivity, which contributes to changes in muscle fibers and atrophy (Patten et al., 2004). For patients with adequate motor control, both resistive and aerobic exercises performed 3–4 times a week for a period of 3 months have been found to improve strength and functional activities. Trials revealed that even when the exercise is initiated years after a stroke, it shows gains with exercise (Saunders et al., 2004).

Recognizing the clinical significance of post-stroke weakness in this population leads to the support of the concept that strength training could be a simple approach for improving motor function and reducing disability, even with the presence of spasticity in the affected limb (Hsu et al., 2002; Clark et al., 2006; Tripp and Harris, 1991). According to Garcia-Cabo a patient with muscle weakness after a stroke can improve strength without negative effects, such as worsening spasticity or hypertonia (García-Cabo and Lopez-Cancio, 2020).

Impaired balance is one of the major problems following a stroke. Moreover, balance impairment has consistently been identified as a risk factor for falls in people with stroke (Batchelor et al., 2012). Balance relies on the complex interaction of neural and musculoskeletal systems, e.g., good balance requires the ability of the muscles, particularly of the lower extremities, to produce adequate force at the appropriate time (Pollock et al., 2000; Carr and Shepherd, 2010b). The balance and stance variables in the present study were significantly improved with centrifugation, depending upon the type of protocol and the side measured. The weight distribution was improved in the normal side with both protocols A and B with no significant differences when combined with exercise (maybe the normal side needed a higher exercise load) but for the paretic side, exercise enhanced the effectiveness when combined with protocol A. The force plate parameters indicated greater levels of significance in the anterior/posterior direction compared to medial/lateral components. For the total range, the longitudinal axis was mainly improved with protocol A combined with exercise. The total CoP displacement showed reduced (smaller variance indicative of improvement in balance abilities) by protocol A (larger COP deflections are associated with less stable balance and in a next step with aging and disease). The average velocity was improved by protocol B combined with exercise. Since balance is important for functional activities such as sitting, sit-to-stand, and walking (Carr and Shepherd, 2010a), decrements in balance performance potentially impair activities of daily living.

To objectively determine the patient's ability or inability to safely balance during a series of predetermined tasks, the Berg Balance Scale test was performed with significant improvement of the score in the rehabilitation period. This balance score is associated with quality of life (Schmid et al., 2013) and is a sensitive (80%) and specific (78%) tool for identifying individuals at risk of falling following a stroke (Maeda et al., 2009). Additionally, the 6MWT was significantly improved from 3 m with a walking aid (cane) to 100 m.

Intermittent artificial gravity *via* centrifugation, a multi-system or integrated countermeasure (Clement and Pavy-Le Traon, 2004), has shown to be beneficial in ambulatory (Evans et al., 2004) and bed-rested subjects (Stenger et al., 2012). Our findings on cardiovascular parameters are similar to previous research studies, which have shown a positive effect of centrifugation with improvement of cardiovascular responses to orthostatic stress (Isasi et al., 2022; Goswami et al., 2015), especially if centrifugation is combined with exercise (Greenleaf et al., 1997; Iwase et al., 2002; Iwase, 2005). In the present study, the improvement of cardiovascular markers over time (Figures 7, 8) showed the effectiveness of the training device in improving the patient's physical condition.

The main mechanism of the effect of AG on the human body in the head-to-toe direction is blood pooling in the veins of the lower part of the body, which may cause a sudden decrease in blood pressure, initiating a stress condition that the cardiovascular system needs to overcome in order to assure blood circulation to all parts of the body. Higher levels of AG increase the stress upon the cardiovascular system and leads to more intense cardiovascular reflex responses in order to maintain blood pressure homeostasis. This leads to inhibition of parasympathetic activity by the autonomic nervous system and increase of sympathetic stimulation triggered by the primary receptors (the arterial baroreceptors in the carotid sinus and the aortic arch areas) for short-term control of the cardiovascular system. We noticed that these responses were more intense when cardiovascular demand increased further due to the combination of SAHC with exercise, and specifically, CO increased significantly to confront the new metabolic demands imposed by the exercise activity (Guyton and Hall, 1996).

Furthermore, muscle activation increased mean systemic pressure due to venous return, and autonomic stimulation increased heart rate and heart contractility. Generally, we noticed a statistically significant effect in all cardiovascular variables between any of our exercise combinations. An increase in heart rate and contractility of the heart acts to maintain the appropriate amount of blood flow to all the organs, in particular the brain. Animal studies have confirmed increased blood flow to the cerebral cortex and brain stem with centrifugation and attribute the changes observed to the central and peripheral vascular systems (Adams et al., 2001). It is likely that the same changes observed in the central and peripheral vascular system, would also take place in the central nervous system vascular interstitial fluid. Rampello et al. (2007) demonstrated that cardiorespiratory training may be better than neurorehabilitation.

Caliandro et al. (2017) demonstrated that stroke may cause changes in small-world characteristics of the cortical network organization as measured by graph theory methodology applied on cortical sources of EEG data. In the present study, significant improvement was revealed in precentral and postcentral gyrus areas after treatment with SAHC alone of combined to exercise, as was identified by graph theory on the cluster coefficient, betweenness centrality metric, and node degree in pre and postcentral gyrus. The cluster coefficient and betweenness centrality metric were affected by both protocols alone, and it seems that exercise added marginally to the final effect, while the node degree presented a significant difference only when SAHC was combined with exercise. The precentral gyrus, located in the frontal lobe and on both sides of the brain, is referred to as the primary motor cortex or as the motor strip. While the planning of movements occurs in the frontal lobe, all the information is processed to the motor strip prior to performance. The left side of the motor strip controls all movement on the right side of body, while the right side controls all movement on the left side. The proper functioning of the motor strip also needs proper development of the muscular, skeletal, and nervous systems to enable a person to move.

A lesion in the right hemisphere, as in the present case, leads to difficulty to shift the body weight between the legs, poor body vertical orientation, body sway, and balance control (Fernandes et al., 2018; Coelho et al., 2019). In the present case, in both right pre- and post-central gyrus, cluster coefficient betweeness and node degree improved significantly after treatment with higher levels when SAHC was combined with exercise, indicating neuron recruitment and neuroplasticity. The effectiveness of the treatment and recovery of neurotransmission after a stroke may be an indication of neuroplasticity (Binkofski and Seitz, 2004; Carmichael et al., 2004). The underlying mechanism may be by enhancing the recruitment of the neurons in both hemispheres of the brain that contribute to performance, increase communication among neurons, and strengthen synaptic connections, especially in the affected side of the brain. This may lead to the improvement of cognitive, language, and motor skills by means of the cerebral processes involved in ordinary learning.

4.1 Mechanism of SAHC alone as a whole-body exercise-mimicking device

Exercise therapy helps stroke patients relearn lost movement patterns. Upper limb functions, muscle strength, balance, and walking ability can be improved. Lower limb training can reduce spasticity, improve strength and endurance, and assist coordination (Kamps and Schule, 2005). Functional neuroimaging studies have shown the evolution of cerebral activity within both hemispheres as patients' skills improve with training and experience (Baron et al., 2004). Centrifugation consists of uniformity and repetitiveness of the exercises at a certain constant pace, which may play an important role in stroke rehabilitation by stimulating the brain's ability to reorganize itself (Plautz et al., 2000). This allows healthy areas of the brain to take over the function of the affected areas. The controlled motion of the uniform centripetal acceleration of the SAHC, a passive movement therapy device, allows exactly this kind of repetitive movement training.

Furthermore, CNS plasticity may be enhanced by muscle activity according to the CNS plasticity model, in part, through the affective signals sent by muscle and joint receptors triggered by movement. The most important is the quality of the movement, which needs to be close to the normal performance as possible and sufficiently repeated. In the case of stroke patients, they may have movements by muscle contractions (Quaney et al., 2009), but they are not normal or sometimes do not exist. There is a need to introduce effective interventions for those stroke patients who are moderately or severely involved. Considering the evidence of CNS plasticity and associated principles of motor learning, we introduced SAHC to mitigate the detrimental effects of bed rest and we mainly focus on the effects of artificial gravity as a whole body physical exercise within a multi-factorial framework in which a variety of lifestyle factors, or pharmacological treatments, are measured and manipulated, taking into account the genetic predispositions for dementia, disease, or cognitive dysfunction (variation in genes, such as the BDNF gene), the efficacy of aerobic exercise in relation to symptom severity, duration of illness, comorbidity of diseases, the brain areas, and molecular factors most affected in the disease, as well as possible interactions with pharmaceutical treatments, and finally, a more refined task manipulations in the context of SAHC intervention will enable a detailed characterization of the cognitive processes that are most affected by artificial gravity and whether such cognitive and neural benefits are transferred to everyday functions.

Stroke patients can train easily and effectively with this device independently of their degree of disability on an individualized training program, lying on a bed with or without additional aerobic or resistive exercise.

The present study validated individualized AG protocols that couple the countermeasure needs of skeletal muscle, bone, and cardiovascular and neurological systems. It also proposed a multi-modal evaluation framework for validating the beneficial role of artificial gravity in the prevention of the physiological deconditioning that normally occurs in microgravity and other bed rest and reduced mobility conditions. Therefore, the SAHC training could be regarded as a new gravity-focused therapeutic approach for rehabilitation interventions.

The present case study shows promising results regarding the utility of the artificial gravity training as a stroke rehabilitation approach. However, forthcoming randomized controlled trials are needed to validate the statistical significance of the results on a large population cohort. The inclusion of either a control group or the comparison with current state of the art approaches is expected to provide concrete scientific evidence. We acknowledge this major methodological limitation of the current approach and aim to address it within the activities of the H2020-funded project, VITALISE No.101007990—https://vitalise-project.eu/ (Bernaerts et al., 2022).

Brain plasticity after stroke refers to the regeneration of brain neuronal structures and/or reorganization of the function of neurons. Not only can CNS structure and function change in response to injury but also the changes may be modified by "activity". For gait training or upper limb functional training for stroke survivors, the "activity" is motor behavior, including coordination and strengthening exercises and functional training that comprise motor learning. The ultimate goal of rehabilitation is to restore function so that a satisfying quality of life can be experienced.

The present case study proposes a novel infrastructure based on the SAHC to investigate the hypothesis that artificial gravity ameliorates the degree of disability resulting from a variety of circumstances from mobility to cognitive integrity and function.

Data availability statement

The raw data supporting the conclusion 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 Bioethics Committee of the School of Medicine of the Aristotle University of Thessaloniki (179/19.03.2020). The patients/participants provided their written informed consent to participate in this study.

Author contributions

CK-P, JV, CF, and PB conceived and designed the research. CK-P and CF conducted experiments. IM, XY, and CF analyzed the data. NK collected the muscle-related data and conducted experiments. ED examined the patient. CK-P and CF wrote the manuscript. CK-P, JV, PB, EK, and CF revised the manuscript. PB and JV supervised the study.

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Conflict of interest

JV is the director and founder of Thirdage LLC.

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.

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphys.2022.1024389/full#supplementary-material

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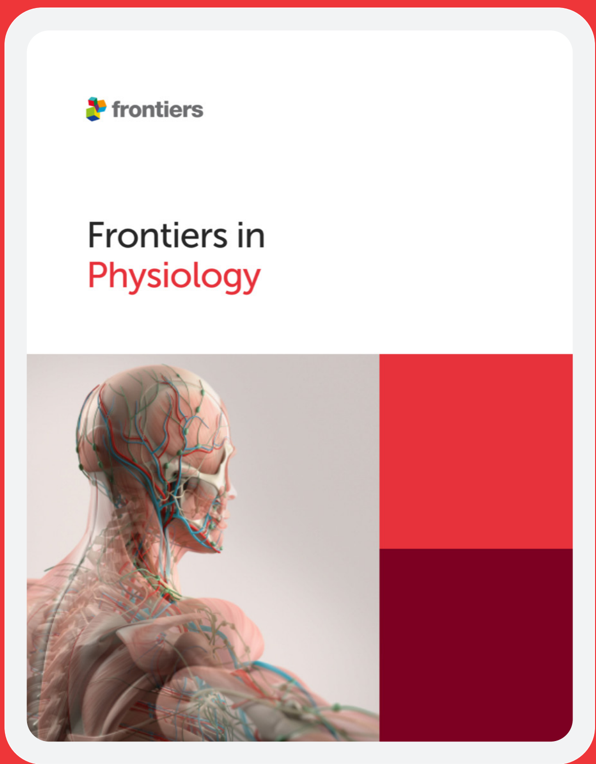

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