System-level Mechanics of Cardio-postural Deconditioning of Older Male and Female Adults in Response to Lower Body Exercises and Immobility

by

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Abstract

Orthostatic tolerance, the capacity to prevent hypotension under gravitational stress, is crucial for both astronauts experiencing microgravity during space missions and individuals facing prolonged periods of inactivity, such as bed rest. This dissertation investigates the complex mechanisms of cardio-postural deconditioning in older adults, with a particular focus on the effects of lower body exercises and immobility on muscle-pump and cardiac baroreflex functions. Employing a 14-day head-down bed rest (HDBR) simulation designed to mimic post-spaceflight conditions and physiological adaptations analogous to those encountered post-hospitalization, this study examines the response of participants aged 55 to 65 to these conditions and a variety of exercise countermeasures. Our research reveals significant differences in the impact of spaceflight-oriented exercises on muscle-pump and cardiac baroreflex activities, with notable variances attributable to sex and the type of exercise intervention. Further exploration into the interactions between cardiovascular and postural stability mechanisms, and the role of individual leg muscles following bed rest, sheds light on the specific contributions of different muscle groups to postural stability and blood pressure regulation. This underscores the necessity of tailored exercise programs that prioritize the strengthening of specific muscles to bolster overall postural control and cardiovascular health. Additionally, the dissertation compares the effectiveness of space-based aerobic exercises and high-intensity interval training in preserving muscle pump baroreflex function following bed rest, providing a thorough analysis of how various exercise modalities aid the recovery and maintenance of this essential reflex. This comparison further highlights the significance of biological sex in determining the outcomes of these exercises. By synthesizing these insights, this dissertation enriches the fields of geriatric healthcare and space medicine, offering innovative perspectives on developing non-invasive, effective countermeasures to maintain cardiovascular and postural health in older adults. These findings have wider implications for fall prevention, rehabilitation programs, and the health of astronauts on long-duration space missions, contributing valuable knowledge towards enhancing life quality for elderly populations and ensuring the well-being of astronauts in challenging environments.

Keywords: Data Mining, Optimization, Signal Processing, Mathematical Modelling, Muscle-Pump Baroreflex; Aging; Cardiac Baroreflex; Data Analytics

Dedication

To my mom, my dad, my wife, and my brother.

Your unyielding belief in me and the unwavering love and support you have showered upon me have been the driving force behind my academic journey

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The success of this dissertation would not have been possible without the support and dedication of many people.

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I owe a great debt of gratitude to Dr. James Wakeling for the constant supply of fresh ideas and insightful remarks that helped shape my research and ultimately led to the successful completion of my dissertation. Because of his guidance and the positive effect he had on my education and career, I owe him a great obligation. In addition, without Dr. David Clarke's help in analyzing different types of data, I would not have been able to complete my dissertation. His never-ending stream of ideas and perceptive critiques has been invaluable to my study and the success of my dissertation.

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I owe a debt of gratitude to every volunteer who agreed to participate in this research and generously dedicated their time to science. Without them, this research would not have been possible.

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List of Acronyms

ANOVA	analysis of variance
AP	anterior posterior
BDC	baseline data collection
BMI	body mass index
BP	blood pressure
B2B	beat-to-beat
CCM	convergent cross-mapping
CIHR	Canadian institute of health research
CIM	center for innovative medicine
CM	countermeasure
CO	Cardiac output
CFN	Canadian frailty network
COP AP	anterior posterior sway
COP ML	medial lateral sway
COPr	center of pressure
COPrv	velocity of COP movement
CSA	Canadian space agency
DBP	diastolic blood pressure
ECG	electrocardiography
EMG	electromyography
EMGimp	Area below EMG envelope within each heartbeat
ESA	European Space Agency
FN	fastigial nucleus

FTA	fraction time active
HDBR	head-down bed rest
HR	heart rate
HF	high frequency
HIIT	high-intensity interval training
IX-BIO4	bipolar three-lead ECG device
LF	low frequency
LG	lateral gastrocnemius
MAP	mean arterial pressure
MG	medial gastrocnemius
ML	medial lateral
MP-BR	muscle pump baroreflex
NTS	nucleus tractus solitarius
PGi	paragigantocellularis nucleus
R	recovery
RI-MUHC	McGill University Health Center Research Institute
S	soleus
SBP	systolic blood pressure
STS	supine-to-stand test
SVR	systemic vascular resistance
TA	tibialis anterior
VLF	very-low frequency
WTC	wavelet transform coherence

Preface

Orthostatic Hypotension (OH), a condition marked by a significant drop in blood pressure upon standing, presents a critical challenge in geriatric healthcare, affecting 20% to 30% of adults over 65. This decline in blood pressure is associated with adverse health outcomes like heart disease, stroke, and increased mortality, exacerbated by aging and various comorbidities. Given its profound impact on the quality of life, understanding and managing OH is essential in geriatric medicine.

This doctoral thesis, conducted at McGill University Health Center in 2021 with support from the Canadian Institutes of Health Research (CIHR) and the Canadian Space Agency (CSA), investigates the effects of inactivity and bed rest on adults aged 55 to 65. We explored the muscle pump baroreflex's response to different exercise countermeasures, including High-Intensity Interval Training (HIIT), aerobic exercises, and strength training, with the aim of mitigating the risks associated with prolonged inactivity, a condition not unlike that experienced by astronauts returning to Earth's gravitational field. Astronauts often experience a form of OH upon re-entry, highlighting the universal importance of this research beyond geriatric populations.

Through this work, we strive to offer novel insights into preventing and managing OH, proposing exercise as a viable intervention to enhance vascular health and improve overall well-being among the elderly and other at-risk groups, such as returning astronauts. This thesis represents a step forward in geriatric and space medicine, offering evidence-based strategies to combat the debilitating effects of OH.

I am deeply grateful for the guidance and expertise of the scientific community that has supported this endeavour, hoping this research will contribute to better health outcomes for those affected by OH and similar conditions in varying environments, including space.

Vancouver, Canada Farshid Sadeghian

Chapter 1. Introduction

1.1 Motivation

Space missions produce adaptations to the effects of microgravity in astronauts , which can have adverse consequences when they return to a gravitational environment, such as landing on gravitational fields like moon and Mars, or returning to Earth [1]. This adaptation leads to the occurrence of syncope, or fainting, among astronauts following their return from the International Space Station, which has been a serious concern for researchers. To counteract the effects of microgravity deconditioning, regular physical activity has been recommended by experts in human spaceflight. However, despite decades of research, the development of an effective, non-invasive, and comprehensive countermeasure to mitigate space-induced cardio-postural deconditioning has only achieved limited success [2].

Interestingly, research has shown that physiological adaptations during spaceflight share similarities with the aging process [3-7]. Aging is often characterized by a decline in physical activity and an increase in sedentary behavior, both of which contribute to the deterioration of physiological systems' functionality [8], and it is projected that by 2050, the number of individuals aged 60 and above will exceed 2 billion [9]. This deterioration can lead to various negative health outcomes, including orthostatic hypotension (OH) [10, 11]. By comprehending the complex mechanisms underlying these physiological processes, we can gain valuable insights into the impact of microgravity on cardiovascular health and discover ways to mitigate the detrimental effects of aging on the cardiovascular system.

The widely used method of six-degree head-down bed rest (HDBR) serves as an experimental model to replicate the effects of microgravity and assess the efficacy of various exercise countermeasures before their implementation in spaceflight [2]. However, it is important to note that there are notable distinctions between spaceflight and HDBR concerning fluid shifts, spinal dysfunction, and radiation exposure, as outlined in Table **1-1** [12]. Several exercise countermeasures have been investigated in HDBR, including aerobic [13, 14] and resistance training protocols [15], novel exercise modalities [16], and combinations of exercise with fluid loading [17], artificial gravity [18-20], or whole-body vibration [15, 21, 22]. Apart from aerospace medicine research, bed rest experimental models also provide valuable insights into the rapid physiological deconditioning experienced during prolonged hospitalization and physical inactivity on Earth. However, it is worth mentioning that most bed rest studies have predominantly focused on young populations, with a median age of 24.5 years [23]. In contrast, the average age of astronauts for their first and last flights is 40.9 years (with a maximum age of 58.8 years) and 45.3 years (with a maximum age of 61.3 years, excluding John Glenn at 77.3 years), respectively [24]. Furthermore, studies involving older adults have rarely incorporated in-bed exercise countermeasures [25]. Consequently, a critical gap exists in knowledge regarding the outcomes of bed rest and exercise countermeasures in middle-aged and older individuals.

Table 1-1. Blood volume changes during spaceflight against those during head-down bed rest (HDBR) (+: increased, -: decreased). Originally published in Diedrich et al. [26]; revised and updated.

	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

The human body comprises different physiological systems, each with its own structure, function, and dynamics. These systems have distinct regulatory mechanisms that allow them to coordinate functions, generate physiological states, and maintain homeostasis [27]. The dynamics of feedback and feedforward mechanisms in systems regulation are illustrated through the cause-and-effect relationships observed in interactions among physiological systems, as evidenced by studies in neural [28, 29], cardiovascular [30, 31], cardio-respiratory [32, 33], and cardio-postural systems [34-36]. Failure to maintain these interactions may disrupt the normal operation of certain systems or set in motion a cascade of malfunctions that ultimately compromises a life-sustaining physiological process [27]. Therefore, understanding the relationships between the many dynamical systems in physiology could aid in tracking their progress and determining their significance.

Previous studies have been constrained by their focus on investigating mechanisms involved in blood pressure regulation separately. For instance, cardiovascular and postural control have been examined independently in the existing literature. Nevertheless, recent studies have demonstrated evidence of the interconnection between these control systems in both healthy young and elderly populations [35-39]. Furthermore, physiological signals, including blood pressure, heart rate, and muscle activity, are generated by intricate control systems and exhibit non-linear and non-stationary characteristics. Unfortunately, the methods commonly employed in the literature to understand and assess the dynamics of these systems rely on Fourier analysis, which assumes stationarity of the signals. This assumption implies that the properties of the hemodynamic fluctuations do not change over time [40]. However, numerous time series, such as physiological signals such as heart rate, postural sway, respiration, and brain activity, contain oscillatory components within a particular frequency band. Therefore, it is essential to employ more suitable and accurate analysis techniques that can effectively capture and characterize the non-stationary nature of these control systems.

Conducting research in this field is crucial for mitigating the risk of fainting episodes among astronauts when they transition from microgravity to a gravitational environment. This is especially significant for long-duration space exploration missions, as prolonged exposure to microgravity can lead to more pronounced deconditioning and difficulties in maintaining normal blood pressure upon re-entry. By comprehensively understanding the factors contributing to cardio-postural deconditioning, we can develop tailored regimens and interventions to safeguard astronauts' orthostatic tolerance and minimize the occurrence of syncope following extended periods of microgravity. The insights gained from investigating these mechanisms have far-reaching implications beyond the realm of space exploration. The development of effective countermeasures and exercise protocols for astronauts can have broader applications in fall prevention and therapeutic programs for elderly individuals and patients who are confined to bed due to illness or injury. This includes individuals dealing with musculoskeletal and cardiovascular conditions, as well as those who are immobile because of stroke, coma, fracture, spinal cord injury, or serious accidents. By leveraging the knowledge and interventions developed for astronauts, we can improve the overall well-being and functional outcomes for these populations, enhancing their quality of life and promoting their recovery and rehabilitation.

1.2 Literature Review

1.2.1 Orthostatic hypotension

The presence of a gravitational field, similar to that on Earth, leads to the accumulation of blood in the lower limb vasculature. The transition from a supine to an upright position leads to an immediate gravitational venous pooling of 500-1000 ml of blood in the lower extremities, splanchnic bed, and pulmonary circulations [27]. Orthostatic hypotension (OH) is a condition characterized by a decrease in systolic blood pressure (SBP) of at least 20 mm Hg or diastolic blood pressure of at least 10 mm Hg upon transitioning from a supine to a standing position [41]. It is particularly prevalent among elderly individuals, affecting approximately 5% of those under 50 years old and 30% of those over 70 years old [42]. Orthostatic hypotension can result in various symptoms, including postural instability, visual impairments, loss of consciousness, and an increased risk of falls [43].

Within the circulatory system during standing, a hydrostatic column is produced, which increases BP as blood descends below the hydrostatic indifference point, which is located a few centimeters below the diaphragm. In terms of magnitude, this effect (75 mm Hg • m-1) is comparable to 'normal' arterial BP (90 mm Hg). The high-pressure gradient prevents blood from returning to the heart because the venous side of the circulatory system is at lower pressures, leading to a decline in stroke volume, cardiac output, and blood pressure [44]. Several strategies are employed to counteract the detrimental effects of this physical phenomenon to maintain consciousness and normal blood flow. These include one-way venous valves, the ability to constrict blood vessels, and the cardio-postural response (Figure **1.1**).

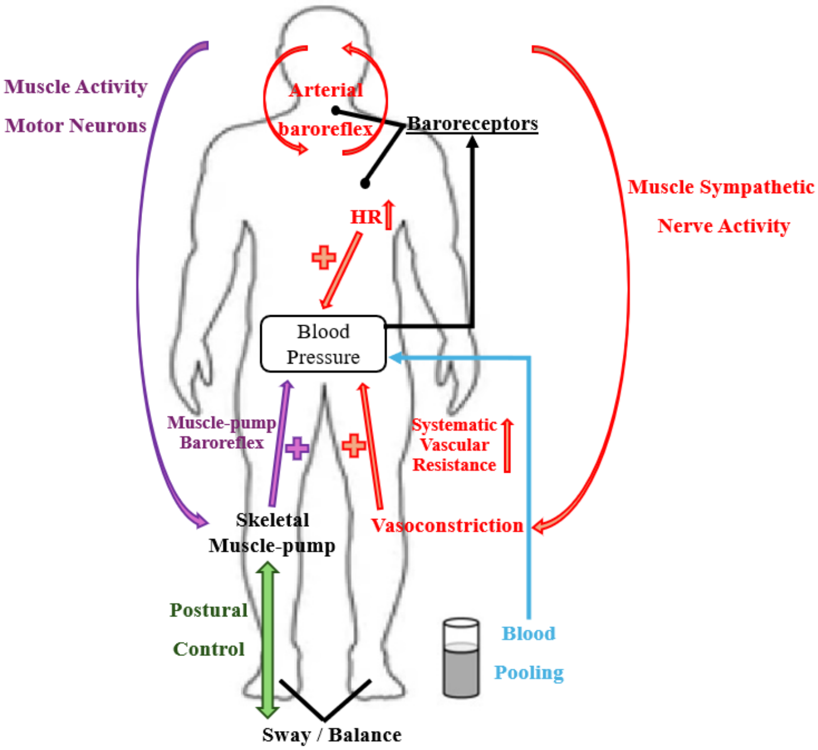


Figure 1.1. Cardio-postural system. Maintaining postural and cardiovascular stability in an upright posture requires complex physiological regulations. Orthostatic stress causes a variety of physiological responses, including the arterial baroreflex, which increases heart rate and peripheral vascular resistance, and the skeletal muscle-pump effect, which propels pooled blood back to the heart.

Complex compensatory physiological mechanisms are activated to restore arterial blood pressure and maintain homeostasis when orthostatic hypotension happens [45-47]. The drop in blood pressure triggers reflex responses initiated by the reduced firing of the baroreceptors to the nucleus of the tractus solitaries (NTS) via the glossopharyngeal and vagus nerves [47, 48]. NTS has further projections that innervate structures such as the nucleus ambiguus, the dorsal motor nucleus, and medulla oblongata. The latter constitutes the starting point of sympathetic motoneurons that will send efferent pathways to the sinoatrial node to increase heart rate and blood pressure. These events result in increased cardiac output (CO), systemic vascular resistance (SVR), and venous return, further increasing CO [47, 48] (Figure 1.2 [36]). However, the arterial baroreflex has limited ability to regulate MAP during orthostatic challenges because of scarce innervation of sympathetic nerve endings in the lower limb veins, which limits its ability to increase venous return [49, 50]. Therefore, standing still in an upright position would cause a significant accumulation of venous blood in the legs.

The collaboration of lower leg skeletal muscles with one-way venous valves plays a vital role in maintaining BP homeostasis through the muscle-pump baroreflex [35]. This mechanism involves the contraction of lower limb skeletal muscles in response to balance correction, propelling venous blood back to the heart and preventing its accumulation in the legs. This increases venous return and, consequently, cardiac output [51-53]. In addition, the maintenance of standing equilibrium is the result of feed-forward control and the integration of sensory information from the visual, proprioceptive, and vestibular systems [54-56]. The central nervous system sends motor responses to lower limb muscles, causing them to contract and ensuring that the center of mass remains within the base of support [55, 57, 58] (Figure **1.2** [36]).

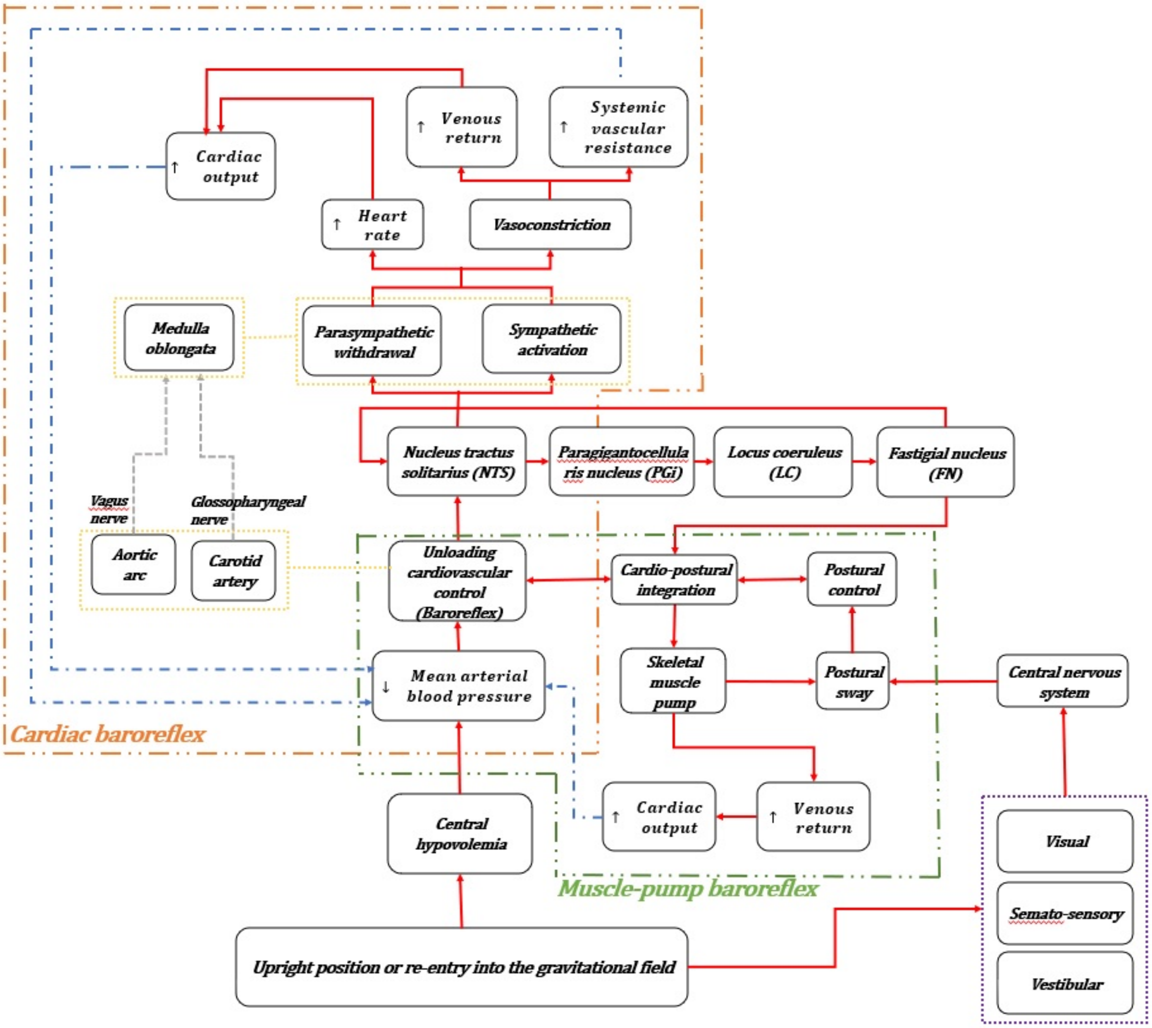


Figure 1.2. Collaborating pathways for regulating blood pressure in an upright position or during astronauts' re-entry into a gravitational field. The diagram illustrates how the cardiac and muscle-pump baroreflexes work together to increase venous return and cardiac output, essential for maintaining blood pressure.

Breathing has also been shown to alter heart rate and blood pressure. Intracardiac pressure drops during inspiration due to the expansion of the thoracic cavity volume. This lowered pressure is also transmitted across the right atrial walls, helping right atrial filling and increasing venous return and BP (so-called respiratory pump) [59-61]. Furthermore, breathing has been found to influence static and dynamic balance via diaphragmatic and ribcage movements, which generate forces that can mechanically displace the COM and cause postural oscillation [62-64]. Breathing can also stimulate the cerebral cortex, affecting lower and upper extremity muscle function and postural balance [65, 66]. Hyperventilation has been demonstrated to reduce blood carbon dioxide levels, resulting in cerebral vasculature constriction leading to dizziness and postural instability, whereas lower limb muscular activity has been shown to compensate for respiratory posture disturbances [67, 68]. Therefore, blood pressure during standing and postural control are regulated not only by the activity of the lower limb muscles but also by respiration.

When a person is in microgravity, the lack of hydrostatic pressure results in an imbalance of forces on the blood column, which ultimately results in a net head-ward influence on blood distribution. As a result, a variety of physiological symptoms, such as, but not restricted to, decreased carotid baroreceptor responsiveness, slowed heart rate, increased stroke volume, increased central blood flow, and decreased total flow, as well as central vein distension and facial edema, were observed. Orthostatic intolerance develops because of a diminished carotid baroreceptor response and reduced total blood volume after spaceflight. On the other hand, OH can arise in older persons from various medical factors, categorized as either neurogenic or non-neurogenic causes. Neurogenic causes can be further classified as primary, which involve a primary disorder affecting the autonomic nervous system, or secondary, which are associated with systemic diseases, leading to autonomic neuropathy [44]. Non-neurogenic causes of OH include reduced intravascular volume, significant decline in cardiac output, and failure of the skeletal muscle pump to effectively return venous blood to the heart. Additionally, certain medications such as tricyclic antidepressants, antipsychotics, alpha-blockers, antiparkinsonian drugs, diuretics, sympatholytics, and vasodilators can affect autonomic function and contribute to the development or exacerbation of OH [69].

1.2.2 Age-Related Changes in Orthostatic Challenge

Numerous physiological changes caused by aging result in structural and functional alterations in the body. Therefore, as we age, it becomes increasingly difficult to maintain a homeostatic balance against stress factors [70]. As with many other systems, orthostatic tolerance mechanisms exhibit age-related declines [71]. The vision and vestibular systems play a crucial role in an upright posture. Deterioration of visual functions associated with aging impacts the perception and activities of older individuals [72]. In addition, diminished in older adults is the vestibulo sympathetic reflex, making it more difficult to sustain arterial blood pressure in an upright position [73]. All three phases of orthostatic challenge are affected by aging [74]: an initial rise in heart rate and decline in blood pressure [75], an early phase of stabilization [76], and a phase of prolonged stabilization [37].

Age-related declines in cardiovagal baroreflex sensitivity result in a deficient response of heart rate to changes in blood pressure. Although not all the underlying mechanisms are clear, arterial stiffness in the baroreceptor-containing segments (such as the carotid artery and aorta), decreased cardiac cholinergic response, and oxidative stress are some of the causes. In contrast to the decline observed in vagal baroreflex sensitivity, no age-related alterations were noted in baroreflex-mediated sympathetic outflow [77]. However, as individuals reach an older age, there is a reduction in the number of pacemakers present in the sinoatrial node. In addition, a decrease in the response to norepinephrine mediated by beta-adrenergic receptors is observed. This decline may be attributed to the downregulation of beta-adrenergic receptors resulting from elevated norepinephrine levels. Another cardiac change associated with aging is a decrease in diastolic filling caused by reduced cardiac compliance and preload. In the absence of compensatory increases in heart rate, a decline in preload can lead to a substantial reduction in cardiac output [71, 78, 79].

In the upright position, the simultaneous increase in peripheral vascular resistance plays a significant role in maintaining blood pressure regulation, in addition to the compensatory cardiac response mediated by the baroreflex. However, another impaired mechanism related to orthostasis in older adults is the diminished vasoconstrictor response to stimulation of alpha 1-adrenergic receptors, accompanied by a lack of the expected rise in peripheral vascular resistance. The exact mechanisms responsible for these changes are not fully understood, but potential causes include decreased vascular compliance because of atherosclerosis, deterioration in the re-uptake and release of norepinephrine, and a reduction in the number of alpha receptors in vascular smooth muscle [80] (see Figure **1.3**).

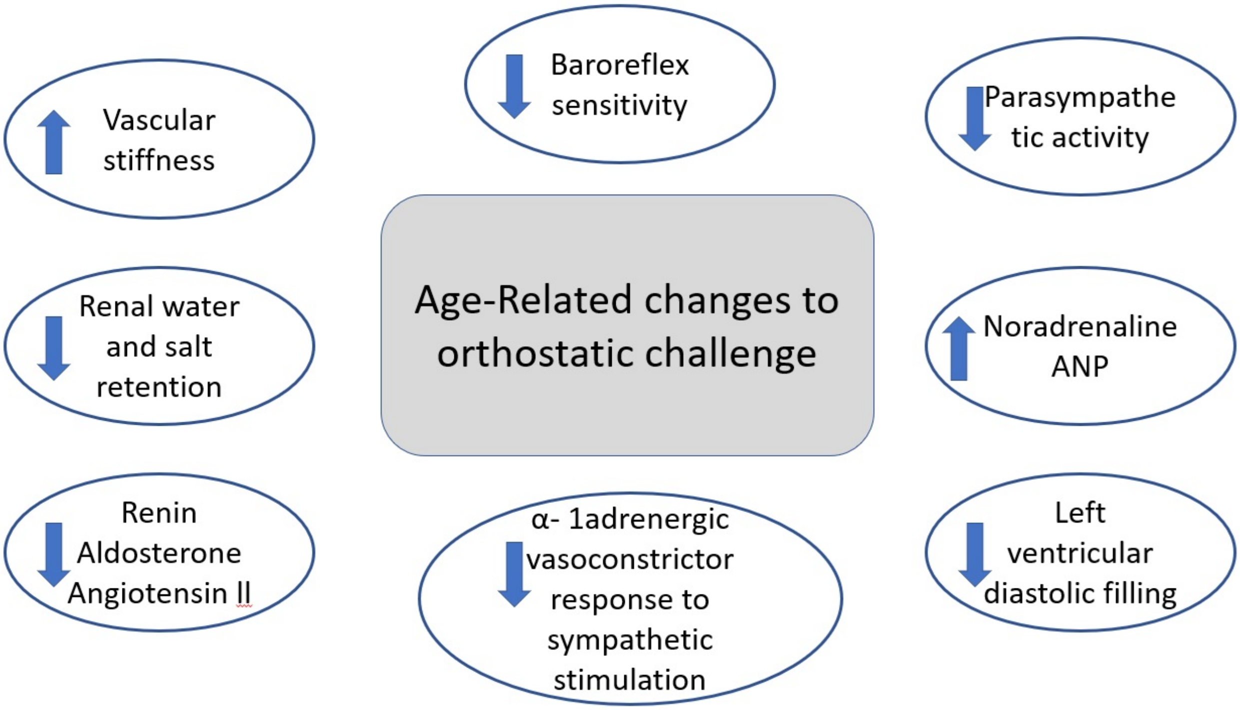


Figure 1.3. Mechanisms of age-related changes during orthostatic challenge [81]

The aging process not only affects autonomic regulation but also impairs skeletal muscle function. In the upright position, aside from autonomic blood pressure control, the lower leg muscles play a crucial role in maintaining blood pressure by actively pumping venous blood back to the heart. However, research indicates that in older adults, muscle pump baroreflexes are diminished compared to younger individuals [82]. These findings have significant implications for the development of targeted exercise or training strategies aimed at mitigating age-related impairments in muscle pump baroreflexes [38]. Other compensatory mechanisms for hemodynamic homeostasis, particularly in long-term maintenance, are neuroendocrine alterations. Nevertheless, it is known that the activity of renin-angiotensin-aldosterone system (RAAS) declines with age. Both the absolute levels and the responsiveness to stimulation of plasma renin and angiotensin II concentrations decline. Nephrosclerosis, decreased renal mass, and impaired juxtaglomerular cell function in the aging kidney contribute to this decline. Furthermore, there is an increase in the levels of atrial natriuretic peptide (ANP), a potent inhibitor of renin and aldosterone secretion, with advancing age (another reason for RAAS inhibition [83]). Additionally, the sense of thirst decreases with age, and the aging kidney exhibits a reduced ability to concentrate urine. Consequently, older adults are more susceptible to dehydration. It is important to note that dehydration is a significant risk factor for orthostatic intolerance [71, 83]. Therefore, orthostatic hypotension is commonly observed in older adults due to the inadequacy of these mechanisms.

1.3 Thesis Outline

In Chapter 2, we provide a comprehensive overview of the key concepts and core methodology that form the foundation of this thesis, including a detailed explanation of the methodologies employed for data analysis. Each subsequent chapter is structured as a standalone publication, with its own introduction and methodology section. These sections briefly outline the motivation behind the specific analysis and the approaches taken to address the research objectives. While the overarching methodology remains consistent throughout the thesis, each chapter incorporates slight variations in methods or data analysis techniques, tailored to the specific goals of that chapter. These differences are clearly described within the respective methodology sections to ensure clarity and coherence for each analysis.

In Chapter 3, we focus on examining the impact of a 14-day head-down bed rest (HDBR) on the maintenance of cardiac and muscle-pump baroreflexes in healthy individuals aged 55 to 65, including both men and women. This chapter investigates the interaction between biological sex and exercise intervention, specifically exploring how they influence the physiological relationship between the cardiovascular and musculoskeletal systems in terms of blood pressure regulation.

Chapter 4 shifts the focus to the relative contributions of individual lower limb muscles to blood pressure regulation and postural control in older adults, both male and female, during standing. The aim is to identify and understand the specific roles of different lower limb muscles in these physiological processes.

In Chapter 5, we compare various exercise modes that target lower leg muscles to evaluate their effectiveness in maintaining muscle-pump function after a period of bed rest. This chapter examines and contrasts different exercise approaches to determine their potential in mitigating the effects of prolonged inactivity.

Finally, in Chapter 6, we summarize the novel findings presented throughout the thesis, discuss the major limitations encountered during the research, and propose potential directions for future investigations to address these limitations.

1.4 Personal Contributions to Data Collection and Analysis

In this dissertation, I was responsible for a broad range of tasks related to conducting experiments, data collection, data mining, analysis, and presenting results in scientific journals and conferences. My contributions can be outlined as follows:

Preparation and Equipment Setup: I ensured the proper setup and attachment of all physiological measurement devices, including EMG, ECG, BP monitors, and force platforms, to participants. This involved verifying power supply compatibility, calibrating signals, and ensuring the accurate functioning of data acquisition software such as LabView, LabScribe, and OTBiolab. I also handled troubleshooting and signal integrity checks before each session to ensure seamless data collection.

Execution of Protocols and Participant Management: I conducted a variety of physiological tests, meticulously adhering to established protocols while monitoring and recording physiological signals during tests such as the supine-to-stand test, vertical jump test, and various exercise protocols (aerobic continuous, aerobic progressive, high-intensity interval training, and lower body strength tests). I managed the attachment of electrodes and participant preparation, ensuring data integrity by maintaining precise control over environmental conditions and enforcing posture and movement guidelines during testing.

Data Handling: I was responsible for the continuous acquisition, accurate capture, and secure storage of all physiological data. This included managing large datasets, maintaining detailed records of each session, real-time monitoring for data quality, and addressing interruptions in data streams as needed.

Data mining and analysis: I independently conducted data mining and analysis of the physiological signals collected during the study, focusing on the last five minutes of the quiet stance phase and across all exercise sessions. After synchronizing, verifying, and filtering the raw data, I segmented signals into beat-to-beat intervals using the Pan-Tompkins algorithm for ECG R peak detection. I derived and analyzed key physiological metrics such as systolic and diastolic blood pressure, RR intervals, and center of pressure. Wavelet transform coherence and convergent cross-mapping were applied to explore causal relationships and trends among cardiovascular and postural control systems, uncovering significant patterns in postural sway, blood pressure, and muscle activity.

Although I was not involved in the original study design, which was completed prior to my joining the Aerospace Physiology Laboratory in 2019, I played a crucial role in the analysis phase. I independently handled all data mining and signal processing to identify meaningful patterns in the physiological data, contributing valuable insights to advance the research objectives.

1.5 Thesis Objectives

This thesis demonstrated the potential of studying the physiological interaction between the mechanisms involved in blood pressure and postural control to prevent orthostatic hypotension associated postural instability and falls in healthy 55- to 65-year-old men and women before and after 14-days of head-down tilt bed rest. Additionally, the thesis aimed to evaluate the cardio-postural contributions of individual muscles during quiet standing, determined which muscle groups benefited the most from a recommended exercise program and how biological sex influenced these outcomes. Furthermore, the impact of different lower body exercises on the activation of lower leg muscles in preserving muscle-pump and cardiac baroreflex function following bed rest was investigated. The major aims of this thesis were summarized below:

- Determining the consequences of 14 Days of Head-Down Bed rest on Muscle-Pump Baroreflex/cardiac baroreflex in older adults.
- Determining HIIT Exercise CM Efficiency Following HDBR on skeletal muscle-pump/cardiac baroreflex responses.
- Studying the impacts of bed rest and proposed exercise CM on male and female regarding muscle-pump/cardiac baroreflex.
- Investigating the Cardio-Postural Contribution of Individual Muscles During Quiet Stand in older adults following HDBR.
- Assessing the efficacy of various modes of exercises CM regarding muscle activation for muscle-pump baroreflex preservation.

1.6Publications

Some of the findings presented in this thesis have been published in peer-reviewed journals, proceedings and presented at international conferences. Other results are under review or in preparation for submission to peer-reviewed journals.

1.6.1 Journal Articles

- Hajj-Boutros G, Sonjak V, Faust A, Balram S, Lagacé JC, St-Martin P, Divsalar DN, **Sadeghian F**, Liu-Ambrose T, Blaber AP, Dionne IJ. "Myths and Methodologies: Understanding the health impact of head down bedrest for the benefit of older adults and astronauts. Study protocol of the Canadian Bedrest Study". Experimental Physiology. 2024 May;109(5):812-27.
- Hajj-Boutros G, Sonjak V, Faust A, Hedge E, Mastrandrea C, Lagacé JC, St-Martin P, Naz Divsalar D, **Sadeghian F**, Chevalier S, Liu-Ambrose T. "Impact of 14 days of bed rest in older adults and an exercise countermeasure on body composition, muscle strength, and cardiovascular function: Canadian space agency standard measures". Gerontology. 2023 Nov 7;69(11):1284-94.
- Blaber AP, **Sadeghian F**, Naz Divsalar D, Scarisbrick IA. "Elevated biomarkers of neural injury in older adults following head-down bed rest: links to cardio-postural deconditioning with spaceflight and aging". Frontiers in Human Neuroscience. 2023 Sep 26;17:1208273.
- Fadil R, Huether AX, **Sadeghian F**, Verma AK, Blaber AP, Lou JS, Tavakolian K. "The Effect of Skeletal Muscle-Pump on Blood Pressure and Postural Control in Parkinson's Disease". Cardiovascular Engineering and Technology. 2023 Dec;14(6):755-73.

- Fadil R, Verma AK, **Sadeghian F**, Blaber AP, Tavakolian K. “Cardio-respiratory interactions in response to lower-body negative pressure”. Physiological Measurement. 2023 Feb 28;44(2):025005.
- Divsalar DN, **Sadeghian F**, Burville K, Tremblay MF, Thomas J, Richter S, Blaber AP. “A spacecraft-compatible combined artificial gravity and exercise (CAGE) system to sustain astronaut health in the next generation of long-term spaceflight”. Journal of Space Safety Engineering. 2022 Dec 1;9(4):577-81.
- **Sadeghian F**, Divsalar DN, Fadil R, Tavakolian K, Blaber AP. “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”. Frontiers in Physiology. 2022 Sep 21;13:943630.
- **Sadeghian F**, Fadil R, Tavakolian K, Blaber AP. “Cardio-Postural Interactions and Muscle-Pump Baroreflex of Individual Leg Muscles Following Bed rest in Older Adults.” (In Progress).
- **Sadeghian F**, Fadil R, Tavakolian K, Blaber AP. “Effects of Space-Based Aerobic and High Intensity Interval Trainings on muscle pump baroreflex in Older Adults Following Bed rest.” (In Progress).

1.6.2 Conference Proceedings and Abstracts

- **Sadeghian F**, Divsalar DN, Fadil R, Taylor C, Stead T, Tavakolian K, Blaber AP. “Exercise during head-down tilt bed rest in older adults: implications for cardiovascular countermeasures”. ACES-CSEW 2022

- **Sadeghian F**, Divsalar DN, Fadil R, Taylor C, Stead T, Tavakolian K, Blaber AP. A. "Effect of Exercise Countermeasure on Muscle-Pump Baroreflex of Individual Leg Muscles After Being Bedridden for Fourteen Days". ICFSR, Toulouse, France, 2023
- **Sadeghian F**, Divsalar DN, Fadil R, Taylor C, Stead T, Tavakolian K, Blaber AP. A., "Canadian aging and inactivity study: effects of 14 days of bed rest on the cardio-postural control in older persons", CSHRN, Calgary, 2023
- Blaber AP**, Sadeghian F**, Divsalar DN, Xu Da, Fadil R, Tavakolian K. "HIIT exercise reduces muscle-pump baroreflex impairment following fourteen days head down tilt bed rest in older adults". NASA-IWS, 2022

Chapter 2. Methodology

2.1 Study Design and Testing Protocols

Canadian Space Agency (CSA) in partnership of Canadian Institutes of Health Research (CIHR) and Canadian Frailty Network (CFN), proposed a new space-inspired exercise program to preserve cardiovascular and musculoskeletal system from deconditioning following 14 days of bed rest. This study was conducted at the McGill University Health Centre (MHUC) Research Institute in 2021. Participants in the HDBR study underwent 26-day bed rest campaigns (Figure 2.1), during which they checked in at the facility five days beforehand to adapt the facilities and perform baseline data collection (BDC). After that, they continuously stayed in six degrees of downward inclination bed rest for 14 days while using a pillow, followed by seven days of recovery (R) to make sure everything had gone smoothly and properly.

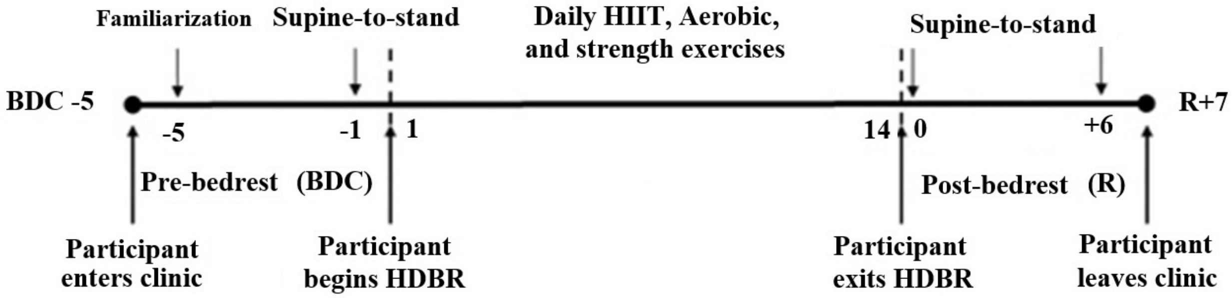


Figure 2.1. Timeline—the participants remained at the testing facility for a total of 26 days, of which 14 days were spent in 6° head-down tilt bed rest (HDBR). Participants arrived at MUHC 5 days prior to entering HDBR. At this time baseline data collection (BDC) was performed. After bed rest, participants 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, R+0, and R+6.

Half of the participants received a specific 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 two weeks of HDBR with 60 to 75 minutes of daily physical activity (Table **2-1**). A detailed description of the exercise protocols is provided by Hedge et al. along with the rationale for their implementation [84]. Briefly, cycling and resistance training with resistive bands were prescribed for muscular and cardiovascular health, resembling high-intensity, low-volume programs [84]. HIIT was added to the exercise program to improve baroreflex responses, aerobic fitness, and cardiovascular health [84]. All exercise sessions were performed in a head-down tilt posture, and their intensities were adjusted based on individuals' heart rates, blood pressures, performance, and tolerance. Apart from the exercise sessions, there were no differences in the standards of care between the two groups.

Table 2-1. Bed rest exercise protocols. A combination of up to three per day was performed with a maximum total time of 62 minutes per day.

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

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

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.

2.2 Participants

Following the screening of volunteers with inclusion and exclusion criteria (Supplemental material), twenty-three participants from the 219 candidates who expressed an interest in taking part, entered the study (Figure **2.2**). 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 bed rest during the recovery phase and were removed from the study before completion. Therefore, data from twenty healthy men and women between 55 to 65 years of age were analyzed (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: male controls (n=5), female controls (n=6), male exercise (n=5), and female exercise (n=4) (Table **2-2**). All participants spent 26 days (5 days of adaptation to the facilities, followed by 14 days of traditional six degrees of downward inclination bed rest in which participants used a pillow, and 7 days of recovery) at the Research Institute of the McGill University Medical Centre (RI-MUHC).

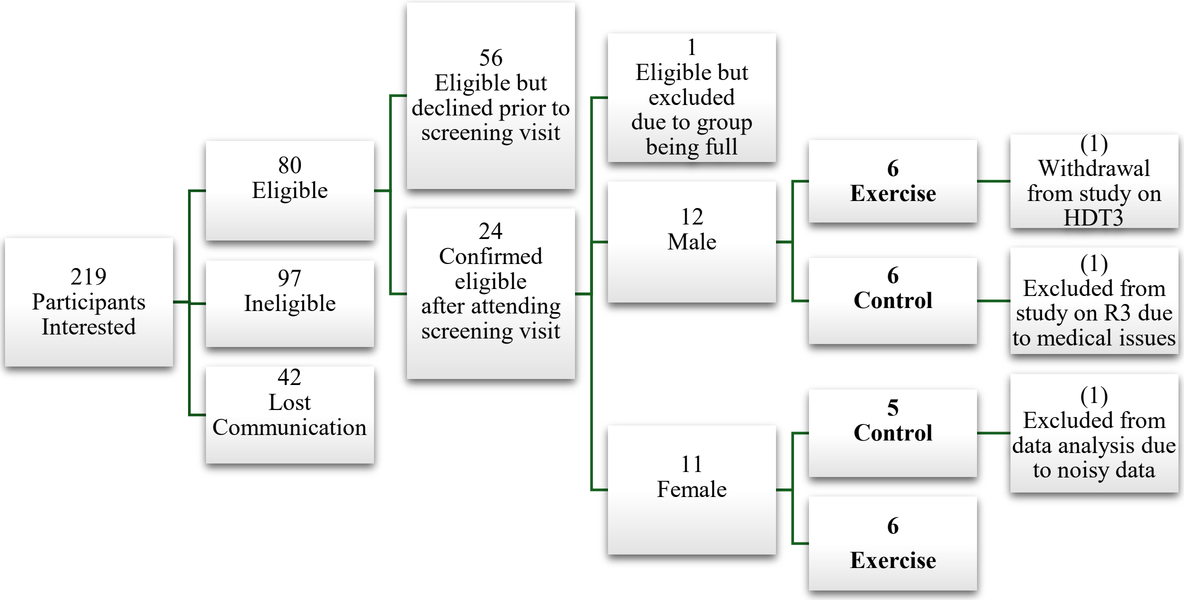


Figure 2.2. Participants selection procedure.

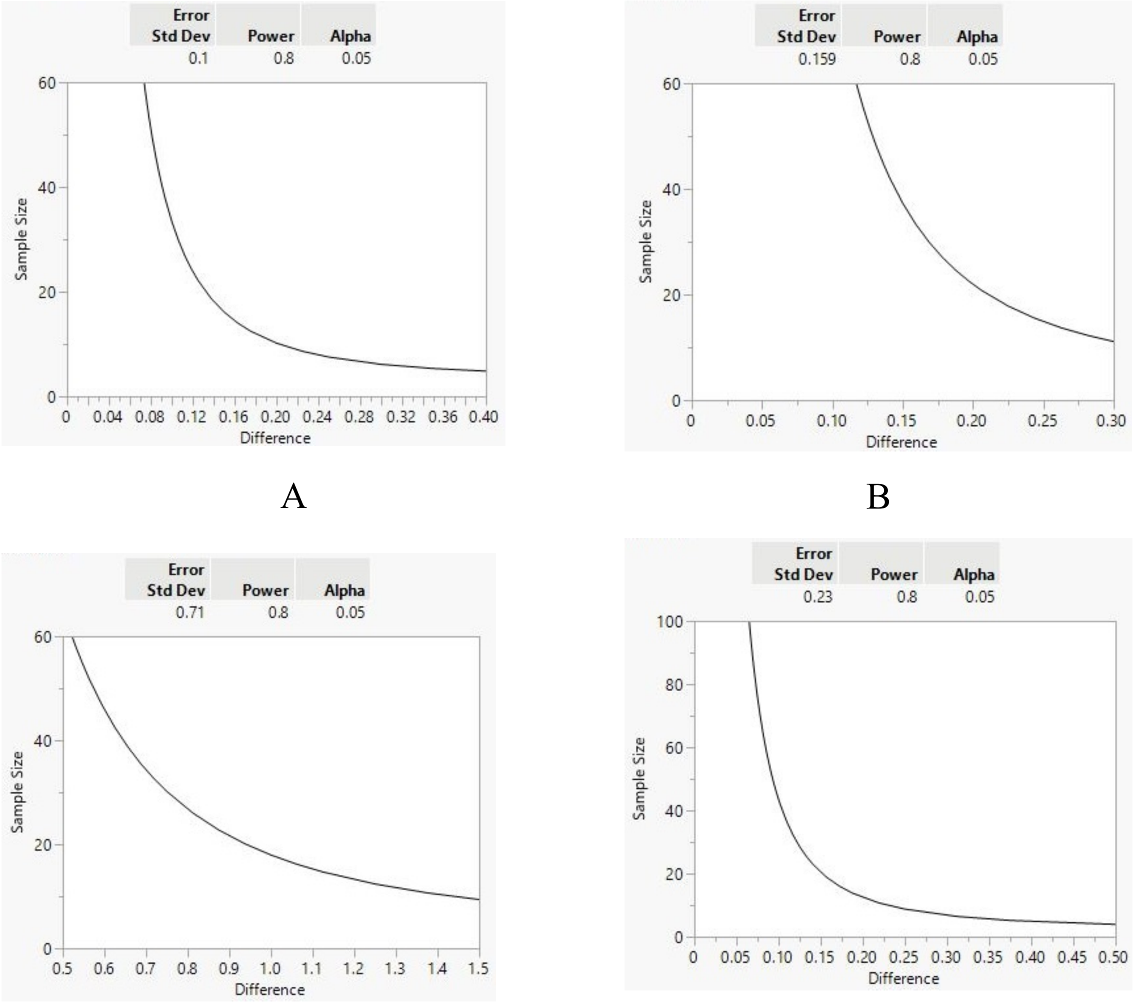
Table 2-2. Participants' anthropometric characteristics in all the studied groups. Originally published in Hajj et al. [85, 86]

Groups	Sex	Age (years)	Height (m)	Body mass (kg)	BMI (kg/m^2)
Control	**F (6), M (5)**	58.4±3.9	166.7±10.7	67.5±14.9	24.0±2.8
Exercise	**F (4), M (5)**	58.4±3.4	167.1±8.4	72.4±13.3	25.7±2.9
In Total	**Either sex**	58.7±0.5	1.67±0.02	70.2±3.2	25.1±2.84

Legend: Mean ± SEM.

2.3 Power and Sample Size Analysis

The CSA protocol anticipated 20–24 participants. We shall determine the power based on a worst-case scenario involving twenty individuals. If we assume that the power to detect a real impact is 0.80 with α = 0.05, we can generate Figure 2.3 for different study aims. Since HDBR affects physiological systems similar to ageing, we anticipate similar muscle-pump baroreflex changes in younger persons after 60-days of HDBR. Based on the LF gain and FTA values derived from Xu. et al [36],we expect to observe 0.1 standard deviation before and after HDBR, with 0.32 μV·s /mmHg differences in LF gain response, which would be satisfied with twenty individuals (Figure 2.3.A). In addition, Figure 2.3.B illustrates that twenty participants are sufficient for detecting individual muscle response variations in FTA, given that the older population had a standard deviation of 0.159 and mean individual muscle response differences of 0.224 [38]. To detect exercise intervention-related responses in our study in muscle-pump baroreflex LF gain it may be necessary to observe a change of 1.13 μV·s /mmHg with 0.71 standard deviation based on Xu et. al. study in 2017 [53], which can be met by twenty people (Figure 2.3.C). In addition, it can be observed from Figure 2.3.D that a sample size of ten individuals for each sex is adequate to identify sex-based variations in FTA, since the older male and female population had an FTA response difference of 0.02 and a standard deviation of 0.23 [38]. Our statistical ability to detect any potential changes in this study would therefore be satisfied with twenty participants (ten in each sex).



A B

C D

Figure 2.3. Power calculation and sample size analyses were performed for four different aims in this study including A) Effects of HDBR on muscle-pump baroreflex LF gain in male and female older adults [36], B) Effect of HDBR on muscle-pump baroreflex FTA of individual leg muscles [38], and C) Exercise intervention-related impacts in muscle-pump baroreflex LF gain preservation following HDBR [53], and D) Sex-related muscle-pump baroreflex FTA responses. All power and sample size analyses were performed using JMP.

2.4 Data Collection

An electrocardiogram (ECG) was recorded with a bipolar three-lead ECG (IX-BIO4, iWorx, USA) in a standard Lead II electrode configuration. The non-invasive Portapres (FMS, Amsterdam, The 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, USA) EMG system. The SENIAM project's [87] suggestions were used to select the locations for EMG sensor placement. Data were collected at 1000 Hz using a National Instruments USB-6218 16-bit data capture equipment and LabVIEW 2013 software (National Instruments Inc, TX, USA).

2.5 Supine-to-Stand Test Procedure

The phases of the study included five days of baseline data collection (BDC), 14 days of HDBR, and seven days of recovery (R) (Figure 2.1). A supine-to-stand (StS) test was administered to activate and assess the cardio-postural control system [37, 53, 88-91] 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 2.1). As the participants had not conducted an StS test during the screening process, the initial test on BDC-5 was considered a familiarization protocol for the participant. Note that StS tests were performed on BDC-1 and R+0 and were conducted one hour after the Canadian Space Agency (CSA) standard tilt test, which had a maximum duration of 15 minutes.

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 minutes. 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 while standing. During the subsequent 6-

minutes 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 [92].

2.6 Data Analysis

The study focused on the last 5 minutes of the quiet stance phase. Data signal processing was completed based on MP-BR analyses established in our previous studies [37, 89, 93, 94]. Before implementing the MP-BR algorithms, the raw physiological data sets were synchronized, verified, and removed noises by filtering. The various physiological signals were then binned into beat-to-beat segments (Figure 2.4), based on the timing of the R peak in the ECG QRS complex. The R peaks were automatically detected using the Pan-Tompkins algorithm [95] and then manually verified. This detection resulted in a time series of heartbeat period (i.e., RR interval). Beat-by-beat time series of SBP were obtained by identifying the maximum pressure values of the BP waveform within each RR interval. The diastolic blood pressure (DBP) time series was constructed by identifying the minimum BP values before the SBP peak of the following beat (Figure 2.4), and beat-by-beat MAP was calculated by averaging the blood pressure waveform between two adjacent DBP valleys. The centre of pressure (COPr) was calculated from medial–lateral sway ($\mathrm{COP_{ML}}$) and anterior-posterior sway ($\mathrm{COP_{AP}}$) (i.e., COPr = $\sqrt{\mathrm{COP}_{AP}^{2} + \mathrm{COP}_{ML}^{2}}$), and the change rate of COPr (velocity of COP movement, COPrv) was calculated as the first derivative of COPr and averaged within each beat, representing the beat-by-beat postural sway velocity (Figure 2.4) [96, 97]. Physiological signals between heartbeats were resampled to 10 Hz using spline-based interpolation before conducting wavelet transform coherence and causality studies.

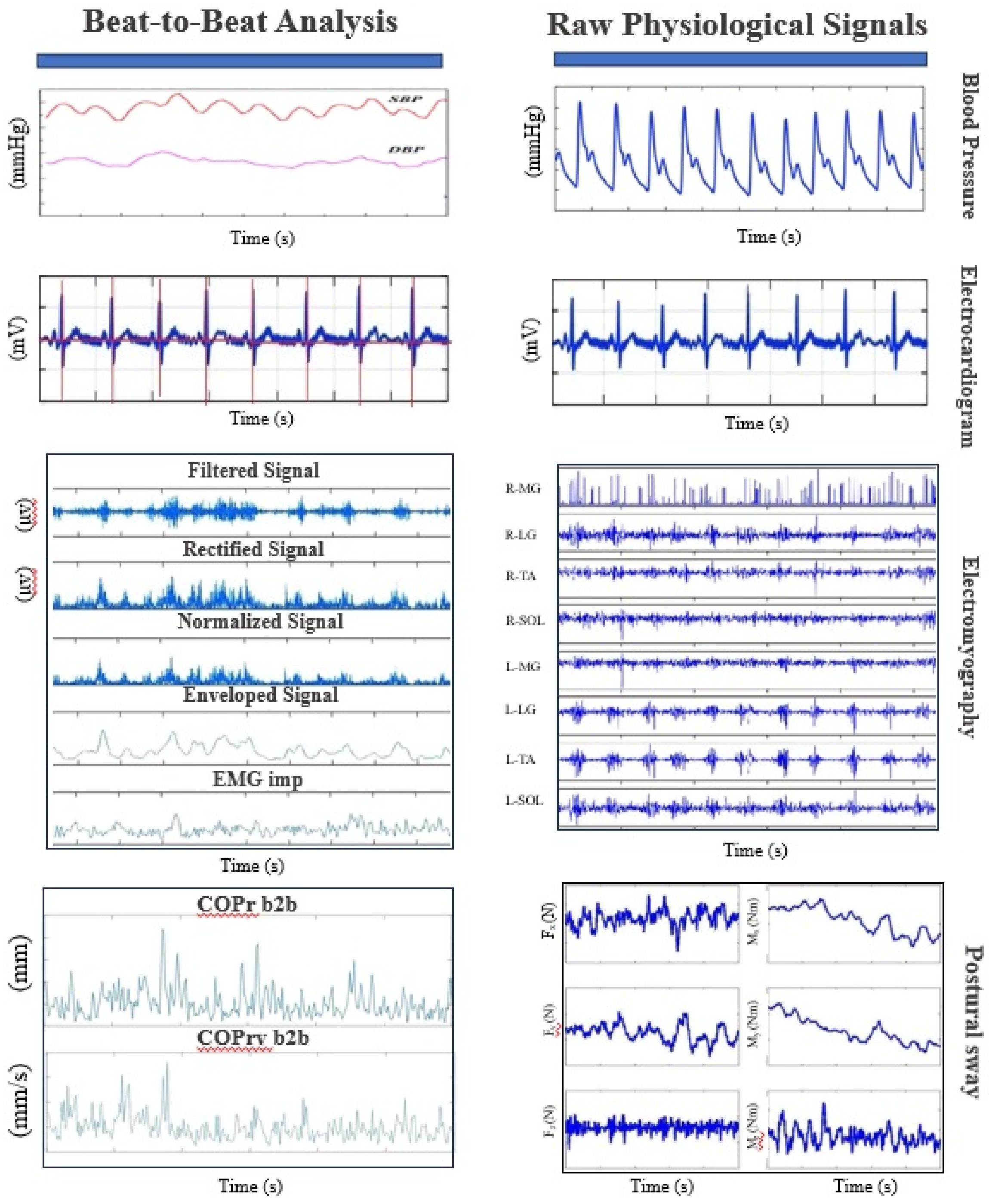


Figure 2.4. Representation of raw and processed physiological signals. The right column illustrates the raw physiological signals captured during the data acquisition phase, reflecting the unprocessed state of the data. The left column presents the results of beat-to-beat analysis, detailing the sequential processing steps undertaken to prepare the EMG signals for advanced signal processing analysis. Legend: BP: blood pressure, ECG: electrocardiogram, EMG: electromyography, R-MG: right medial gastrocnemius, R-LG: right lateral gastrocnemius, R-TA: right tibialis anterior, R-SOL: right soleus, L-MG: left medial gastrocnemius, L-LG: left lateral gastrocnemius, L-TA: left tibialis anterior, L-SOL: left soleus, SBP: systolic blood pressure, DBP: diastolic blood pressure, COPr: center of pressure, COPrv: velocity of COP movement.

2.6.1 Electromyographic Data Interpretation

EMG data interpretation relies on the precise placement and configuration of electrodes. In this study, the Bagnoli-8 surface EMG system (Delsys Inc, MA, USA) was employed to record signals from four bilateral lower leg muscles: the tibialis anterior, lateral soleus, and medial and lateral gastrocnemius. Each EMG sensor features internal shielding to reduce external electrical noise and uses 99.9% pure silver bars (10mm long and 1mm in diameter) spaced 10mm apart for optimal signal consistency. The sensors' curved design enhances skin adhesion and minimizes the negative effects of sweat during exercise. For accurate signal detection, sensors were aligned in parallel with the fiber orientation of the underlying muscle and positioned centrally on the muscle belly, avoiding tendons and edges. To ensure high-quality data collection, the Delsys Adhesive Sensor Interface was used to secure the sensors to the skin, minimizing motion artifacts and line interference. Prior to sensor application, the skin was prepared by removing excess hair and cleaning with alcohol to eliminate oils and residues, followed by thorough drying.

Two primary indirect effects can impact the accuracy of surface EMG recordings. Firstly, varying the inter-electrode distance during muscle contraction can distort the action potentials' amplitude, shape, and width, thus affecting the EMG signal's amplitude and frequency characteristics. To mitigate this, sensors with a fixed inter-electrode distance made from lightweight materials were utilized. Second, movement of the electrodes and cables due to cable pull or inertia can induce artifacts by destabilizing the electric layer and altering impedances. To prevent this, cables were secured with tape and elastic fabric wrap. Besides, standardized electrode placement using anatomical landmarks as recommended by the SENIAM project (Surface Electromyography for the Non-Invasive Assessment of Muscles) was implemented to ensure consistent EMG signal reliability across different test days. Repeatability and reliability were verified by comparing results from the two pre-HDBR StS tests. A regular Student's t-test with a Bonferroni adjustment confirmed no significant changes in the mean value of filtered, rectified, normalized and enveloped EMG signals within participants and between sexes and intervention groups across these two days (Appendix Table 1). These controls ensured that observed differences in EMG signals pre- and post-bed rest were due to physiological changes rather than measurement artifacts.

The interference pattern of EMG is of random nature, due to the fact that the actual set of recruited motor units constantly changes within the diameter of available motor units and the arbitrary way the motor unit action potentials superpose. This results in the fact that a raw EMG burst cannot be reproduced a second time by its precise shape. To address this problem, the non-reproducible part of the signal is minimized by applying digital smoothing algorithms that outline the mean trend of signal development. The steep amplitude spikes are cut away; the signal receives a “linear envelope”. Two algorithms are established:

- Moving average: Based on a user defined time window, a certain amount of data are averaged using the sliding window technique. If used for rectified signals it is also called the Average Rectified Value: and serves as an “estimator of the amplitude behavior” (SENIAM). It relates to information about the area under the selected signal epoch.
- Root Mean Square (RMS): Based on the square root calculation, the RMS reflects the mean power of the signal (also called RMS EMG) and is the preferred recommendation for smoothing.

A low-pass filter serves as an effective alternative to Moving Average and RMS smoothing for creating a linear envelope EMG [98]. In this study, the SENIAM project recommendations were followed, leveraging the collective expertise of 16 European groups specializing in surface EMG development and application to facilitate effective data exchange and clinical knowledge sharing. To address the interest in cardio-postural interactions below 0.5 Hz, a higher-order Butterworth filter was set at 5 Hz (the lower limit of the SENIAM recommendation). This filter offers notable advantages, including sharper cutoff characteristics that enable precise control over the retained frequency components. Moreover, their recursive application significantly reduces or eliminates phase shift, a common issue that can cause delays or distortions in signal timing. By effectively attenuating high-frequency noise while preserving essential lower-frequency components, the low-pass filter produces a smooth and interpretable linear envelope of the EMG signal, enhancing its utility in both research and clinical applications.

As surface EMG sensors can lose their connection during testing, they may record noise instead of muscle activity, so manual validation was required before evaluating the specific muscle EMG data. To facilitate comparisons within the same muscle across different days, between individuals, and between muscles, normalization based on peak activation levels during maximum contractions was performed in each test. Rectification and smoothing techniques minimized noise and artifacts, with the area under the rectified EMG envelope between successive heartbeats used to calculate the beat-to-beat EMG (EMGimp) of each muscle. Besides having bilateral values represented by four muscle groups, rectified EMG signals from individual leg muscles (LG, MG, TA, SOL) were summed to obtain aggregate EMG to represent overall muscle activity [37, 89]. EMG recordings from four separate muscles groups or from the summed EMG were used to determine a relationship between muscle activity and BP. We combined the bilateral EMG values of all the muscles regardless of which leg was weight-bearing during standing, since our focus was on examining how BP interacts with muscle activity to maintain orthostasis while standing. This may involve one leg supporting the body weight for a period of time, followed by a shift to the other leg, while the other leg is in a relaxing position. As a result, muscles were working together to maintain posture and regulate BP.

2.6.2 Wavelet Coherence Transform

Physiological signals, such as blood pressure, heart rate, muscle activity, and postural sway, are produced by intricate control systems. Therefore, it is crucial to comprehend the behavior of these systems, including trends, periodicities, and the coherence among the representative signals they generate. In order to characterize these systems and evaluate their dynamic changes, Fourier analysis-based methods have been devised. However, these methods often assume that variations in cardiovascular and postural hemodynamics are stationary, presuming that the statistical properties of these systems remain constant over time [40]. Consequently, more suitable analysis techniques are necessary to capture the natural non-stationary aspects of the cardiovascular and postural control systems. Continuous wavelet transform (CWT) has been employed to examine physiological signals in the time-frequency domain [38, 40, 99, 100]. While CWT is a commonly used tool for analyzing localized oscillations in a time series, wavelet transform coherence can be utilized to investigate the presence and strength of coherence between two time series in the time-frequency domain.

The CWT is a technique that breaks down a time series in the time-frequency domain. This is achieved by sequentially convolving the time series with a mother wavelet function ψ_0. This wavelet function is stretched over time by adjusting its scale (s) [101]. In this study, we employed the Morelet wavelet ($\omega_0 = 6$), which is defined as:

$$\psi_0(\eta) = \pi^{-\frac{1}{4}} e^{i\omega_0\eta} e^{-\frac{\eta^2}{2}} \qquad (1)$$

The continuous wavelet transform of a time series X of length N with values x_n ($n = 1, \ldots, N$) sampled from a continuous signal at a time step of Δt is defined as:

$$W_n^X(\mathrm{s}) = \left(\frac{\Delta t}{s}\right)^{\frac{1}{2}} \sum_{n'=1}^{N} x_{n'}\, \psi_0^* \left[(n - n')\frac{\Delta t}{s}\right] \qquad (2)$$

where s is the scale-changing stretch parameter, n denotes the time-sliding translation parameter used to slide the wavelet function in time, and $*$ indicates the complex conjugate [102]. The high-frequency components of a signal are related to smaller scales, and the low-frequency components to larger ones. The wavelet power spectrum [40] of a time series X with values x_n is defined in a manner similar to that of Fourier analysis:

$$W_n^{XX}(s) = W_n^X(s)\, W_n^{X^*}(s) = |W_n^X(s)|^2 \qquad (3)$$

Given two time series X and Y with values x_n and y_n and wavelet transforms $W_n^X(s)$ and $W_n^Y(s)$, the cross wavelet transform (XWT) of X and Y is defined as:

$$W_n^{XY}(s) = W_n^X(s)\, W_n^{Y^*}(s) \qquad (4)$$

Where * denotes the complex conjugate.

The cross-wavelet power between X and Y is defined as $|W_n^{XY}(s)|$ and reveals areas with high common power, while the complex argument of $W_n^{XY}(s)$ represents the relative phase between X and Y [102].

The gain between two time series X and Y can be expressed as:

$$G_n^{XY}(s) = \left|\frac{W_n^{XY}(s)}{W_n^{XX}(s)}\right| \qquad (5)$$

The localized correlation coefficient between two time series X and Y in the time-frequency domain is quantified by the squared cross-wavelet coherence $\mathrm{R}_\mathrm{n}^2(\mathrm{s})$, which varies from zero to one. Here we define the wavelet coherence as the square of the cross-wavelet coherence.

$$\mathrm{R}_{\mathrm{n}}^{2}(\mathrm{s}) = \frac{|\langle s^{-1}W_n^{XY}(s)\rangle|^2}{\langle s^{-1}|W_n^X(s)|^2\rangle\langle s^{-1}|W_n^Y(s)|^2\rangle} \tag{6}$$

Where $\langle\,.\,\rangle$ is a multidimensional smoothing operator in time and scale dimensions. Using a weighted running average in the time and scale directions, as described in [101], we can smooth out the wavelet power spectra and remove the singularities, highlighting the regions of significant power. Furthermore, a Monte Carlo simulation with implementing the first-order autoregressive (AR1) model and a large ensemble of surrogate data set pairings with the same coefficients as the original input data pair, the statistical significance threshold of $\mathrm{R}_{\mathrm{n}}^{2}(\mathrm{s})$ can be estimated [102].

2.6.3 Convergent Cross Mapping

The human body is composed of numerous interconnected systems (cardiovascular, respiratory, nervous, skeletal, and postural, etc.) that work together to enable us to regularly breathe, eat, sleep, and move. To comprehend the behavior and function of individual systems, it is crucial to comprehend the interaction between multiple components and the cause-and-effect relationships they generate. Failure to maintain this interdependence and interaction may interfere with the function of an individual system or contribute to the dysfunction of a vital physiological system, such as the cardiovascular, respiratory, or renal system. Therefore, knowledge of cause-and-effect relationships between physiological systems can be used to evaluate the function of these systems and monitor their performance.

To identify causal interactions between linear and stationary stochastic dynamic systems, the Granger causality method has been extensively utilized. In situations where both the cause and effect have deterministic dynamics, Granger causality is unable to extract causal information. The application of Granger causality is further constrained by the assumptions of linear statistical inference, stationary signal behavior, and proper model order selection. Granger causality's constraint of linearity is thought to be addressed by the transfer entropy method; however, its application is limited to stationary signals, and it is also necessary to estimate the probability density function of the considered signals. Since physiological signals exhibit inherent nonlinear behavior, a nonlinear technique would be required to draw precise conclusions about the complex dynamics of the causal interaction between physiological systems.

Convergent cross mapping (CCM) is a non-linear technique for measuring the bidirectional causal relationship between two time series X $(\mathrm{x_t}, \mathrm{t} = 1, \ldots, \mathrm{L})$ and Y $(\mathrm{y_t}, \mathrm{t} = 1, \ldots, \mathrm{L})$, where L is the length of the time series. To infer causation, CCM uses state-space reconstruction (system state representation using successive lags of a single time series) and Takens' Theorem, which stipulates that if X causes Y, then the historical values of X can be reconstructed from the variable Y alone. Practically speaking, this is achieved via the "cross mapping" technique: a time delay embedding is generated from Y, and the ability to estimate X from this embedding reveals how much information about X has been encoded into Y. As a result, how efficiently Y cross-maps X determines the causal effect of X on Y [103, 104].

To do so, the lagged coordinates of variables X and Y are employed to generate the shadow manifolds of $\mathrm{X(M_X)}$ and $\mathrm{Y(M_Y)}$. The lagged coordinates of X $(\tilde{\mathrm{x}}_\mathrm{t})$ and $\mathrm{Y}(\tilde{\mathrm{y}}_\mathrm{t})$ are constructed [105, 106] as:

$$\tilde{x}_t = (x_t, x_{t-\tau}, x_{t-2\tau}, \ldots, x_{t-(E-1)\tau}) \tag{7}$$

$$\tilde{y}_t = (y_t, y_{t-\tau}, y_{t-2\tau}, \ldots, y_{t-(E-1)\tau}) \tag{8}$$

Where $t = 1 + (E - 1)\tau, \ldots, L$, E is the embedding dimension, and τ is the time lag. Each of the vectors $\tilde{x}_t$ and $\tilde{y}_t$ represents a point in the E −dimensional space. The reconstructed M_X

and M_Y manifolds are represented by the collection of vectors $\{\tilde{x}_t\}$ and $\{\tilde{y}_t\}$, respectively. The next step is to find the minimum $E + 1$ nearest neighbors of each $\tilde{x}_t$ in M_X. Let's note the time indices (from closest to farthest) of the $E + 1$ nearest neighbors of $\tilde{x}_t$ by $t_1, t_2, \dots, t_{E+1}$. The nearest neighbors of $\tilde{x}_t$ in M_X are used to estimate Y.

$$\hat{Y} \Big| M_X = \sum_{i = 1}^{E+1} w_i \, y_{t_i} \tag{9}$$

With $w_i = u_i / \sum_{j=1}^{E+1} u_j$, $u_j = \exp\,[-d(\tilde{x}_t, \tilde{x}_{t_j}) / d(\tilde{x}_t, \tilde{x}_{t_1})]$, and $d(\tilde{x}_t, \tilde{x}_s)$ is the Euclidean distance between the two vectors $\tilde{x}_t$, and $\tilde{x}_s$. Predicting Y by M_X is equivalent to Y causing X, and the strength of causality flowing from Y to X is quantified by calculating the Pearson correlation coefficient between the original time series Y and the estimated $\hat{Y}|M_X$. Similarly, to know if X is causing Y (cross mapping of X by using M_Y: $\hat{X}|M_Y$), we can calculate the Pearson correlation coefficient between X and $\hat{X}|M_Y$.

Chapter 3. Spaceflight-Inspired Exercises During Head-Down Tilt Bedrest Blunted Reductions in Muscle-Pump but not Cardiac Baroreflex in Older Persons[1]

3.1 Summary

As part of the first Canadian aging and inactivity study (CAIS) we assessed the efficacy of space-based exercise countermeasures for the maintenance of cardiac and muscle-pump baroreflexes in older persons on 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, were 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 six minutes after HDBR, with six of those being female. Our findings showed that two weeks of bedrest differently impaired skeletal muscle's ability to return blood to the venous circulation in various sexes and intervention groups. Comparing values after bedrest with before bedrest values, there was a significant increase in heart rates (Δ of +25%; +17% in MC

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[1] https://doi.org/10.3389/fphys.2022.943630

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 changes depended 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 the female control, showed parallel decreases in cardiac active gain and causality. The FC group had an increase in cardiac causality, despite a similar decline in cardiac active gain. Results showed that the proposed exercises might alleviate muscle-pump baroreflex declines but would not influence the cardiac baroreflex decline from 14 days of inactivity in older adults.

3.2 Background

The elderly population is increasing globally [107], and it is predicted that the number of individuals over the age of 60 will surpass 2 billion by 2050 [9]. Aging is often characterized by a decrease in physical activity and an increase in sedentary behavior. Both changes in lifestyle aggravate the lack of functionality in physiological systems [8], which can result in deterioration of overall functional health, such as through orthostatic hypotension [10, 11]. Concerns about increased rates of sedentary lifestyles in older people became 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 [3-7].

Spaceflight-induced weightlessness is known to reduce muscle size and strength and cause functional changes in 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 [108, 109]. These multi-system changes can negatively affect the astronaut's ability to perform mission-related tasks and increase the risk of loss of conscious 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 [110]. 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. [84].

On board the international space station astronauts use three different types/modalities of exercise equipment; cycle ergometer, treadmill, and advanced resistive exercise device (ARED) [111]. 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 [111, 112]. 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 [113, 114]. 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 [111].

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 to establish a treatment/prevention program [1, 109]. 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 [115]. 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. [1, 109, 116].

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 ten days of bedrest in older persons induced remarkable muscle weakening, including a loss in whole-body lean mass (−1.50 kg; $P = .004$), lower extremity lean mass (−0.95 kg; $P = .003$), and strength (−19 N·m·s-1; Δ of −15.6%; $P = .001$) which is significantly greater than seen annually in the average aging population [117]. Furthermore, six-degree head-down bedrest (HDBR) has been shown to be an effective analogue of microgravity/spaceflight conditions in order to simulate cardiovascular and musculoskeletal systems' deconditioning [118, 119]. 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 [84] during 14 days of 6-degree head-down tilt bedrest (HDBR) on maintaining the cardiac and muscle-pump baroreflexes in healthy 55 to 65 year old men and women. 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 [36].

Our team has developed a series of techniques to study the significance of lower limb muscle activities in maintaining BP. For this purpose, we have adapted the wavelet transform coherence (WTC) analysis [37, 53, 89] and convergent cross mapping (CCM) causality [91, 120, 121] 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 blood pressure muscle-pump activity 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.

3.3 Data analysis

In this study, we report the results from the stand portion of the StS test. The data analysis process includes data preprocessing, wavelet transform analysis, convergent cross mapping, and statistical analysis to examine the impact of HDBR, sex, intervention, muscle groups, test days, and their interactions on the measured response variables of muscle-pump and cardiac baroreflex.

3.3.1 Data preprocessing

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 participants had their feet in the proper position, they were facing directly forward, and were standing free of assistance, the stand clock was started. We analyzed the first 180 s of the stand to examine the reflex responses immediately following the transition period. Subsequently, the cropped data of every experiment part was filtered to draw out non-representative frequencies and to prevent aliasing, while preserving the most significant harmonics or the harmonics of specific interest [122]. The BP and ECG signals were both low-pass filtered with a zero-phase eighth-order Butterworth filter with a cut-off frequency of 20 Hz [122-124]. The EMG signals of every electrode were also low-pass filtered but with a slightly different filter, a zero-phase second-order Butterworth filter with a cut-off frequency of 5 Hz [53, 125]. This cut-off frequency was chosen because the frequency response of the muscle-pump baroreflex is typically located in the low frequency domain, below 0.5 Hz, of the EMG signal [88, 126, 127]. After executing the filtering, the QRS complex was detected based on the ECG signal by using the Pan-Tompkins algorithm [128]. This algorithm can detect 99.3% of the QRS complexes correctly, which is therefore followed by a manual check to ensure the correct detection of every QRS complex. The detected R waves are then used to calculate the interval between two consecutive R waves (i.e. RR interval), which provides a time series of all heartbeat periods within the analyzed interval. Based on this time series, both the BP and EMG signal can be segmented into a beat-to-beat signal vector, which contains separate signal chunks of each heartbeat in the analyzed interval. 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. Then, the filtered beat-to-beat EMG signals of the four individual electrodes (TA, SL, MG and LG) were added together to create an aggregate beat-to-beat EMG signal for each leg. This aggregated signal represents the overall muscle activity in each leg, which is justifiable considering the interest to evaluate the activation of the muscle-pump baroreflex as a whole and not region specific [37, 89]. First, the mean of aggregated EMG signal was calculated for every beat, which will be referred to as EMGtotal. Second, the area under the curve (AUC) of the EMG signal was calculated for each beat, referred to as EMGAUC, which illustrates the strength of a muscle contraction within a certain beat. Besides the strength, this parameter also incorporates the duration of the contraction, since a short and strong contraction will deliver the same result as a long and weak contraction. As a result, this parameter is considered to deliver a truthful representation of the relative amount of muscle contraction within a heartbeat. Before wavelet transform coherence and causality studies, beat-to-beat physiological signals were interpolated using the spline approach and resampled to 10 Hz.

3.3.2 Wavelet Coherence Transform

A Morlet wavelet was used to produce time–frequency distributions for the signal pair SBP → EMGimp (muscle-pump baroreflex) and SBP → RR (cardiac baroreflex) [37, 89]. Monte-Carlo simulation was used to determine the significant coherence threshold [53]. 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 [129] 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 [102] and averaged over sections of significant WTC within each frequency range. The effectiveness of each interaction was further described using 'Active Gain', (Gain × FTA) [36].

3.3.3 Convergent cross-mapping

The convergent cross-mapping technique was used to calculate the causal relationship between the signal pairs (EMGimp and SBP) and (RR and SBP) [105]. Details on the methods may be found in Verma et al. [91] and Sugihara et al.'s supplemental material [105]. 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.

3.3.4 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 [130].

Given the small numbers of participants in each group (n=4 to 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. [131]. 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 [132], and data are reported as significant ($p<0.05$) or trends ($0.1>p\geq 0.05$).

3.4 Results

3.4.1 Presyncope

Seven of the twenty participants were unable to complete the StS test on R+0 (Table **3-1**). 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 six-minute 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 **3-1**) reducing the analysis sample size for the female exercise group on R+0 to five.

Table 3-1. Bedrest exercise protocols. A combination of up to three per day were performed with a maximum total time of 62 minutes 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

3.4.2 Cardiovascular and electromyography responses

The cardiovascular and EMG measurements were influenced considerably by 14-day 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 3-2), then responses between groups were compared using changes in values from BDC-1; increases being positive and decreases being negative (Figure 3.1 and Figure 3.2).

During the quiet stand of the R+0 StS test, 2 hours 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 **3-2**, Figure **3.1**), with a significant reduction towards baseline values on R+6 in female (p=0.019), but not the male participants (Figure **3.1**).

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 **3-2**, Figure **3.1**). No change from baseline was observed with the female exercise group on R+0 or any group on R+6 (Table **3-2**); however, significant reversals from R+0 occurred with the female control and male exercise SBP responses to standing. (Figure **3.1**). No changes from baseline were found for DBP or MAP for all groups studied (Table **3-2**); however, similar to SBP, there were trend reversals from R+0 to R+6 (Figure **3.1**).

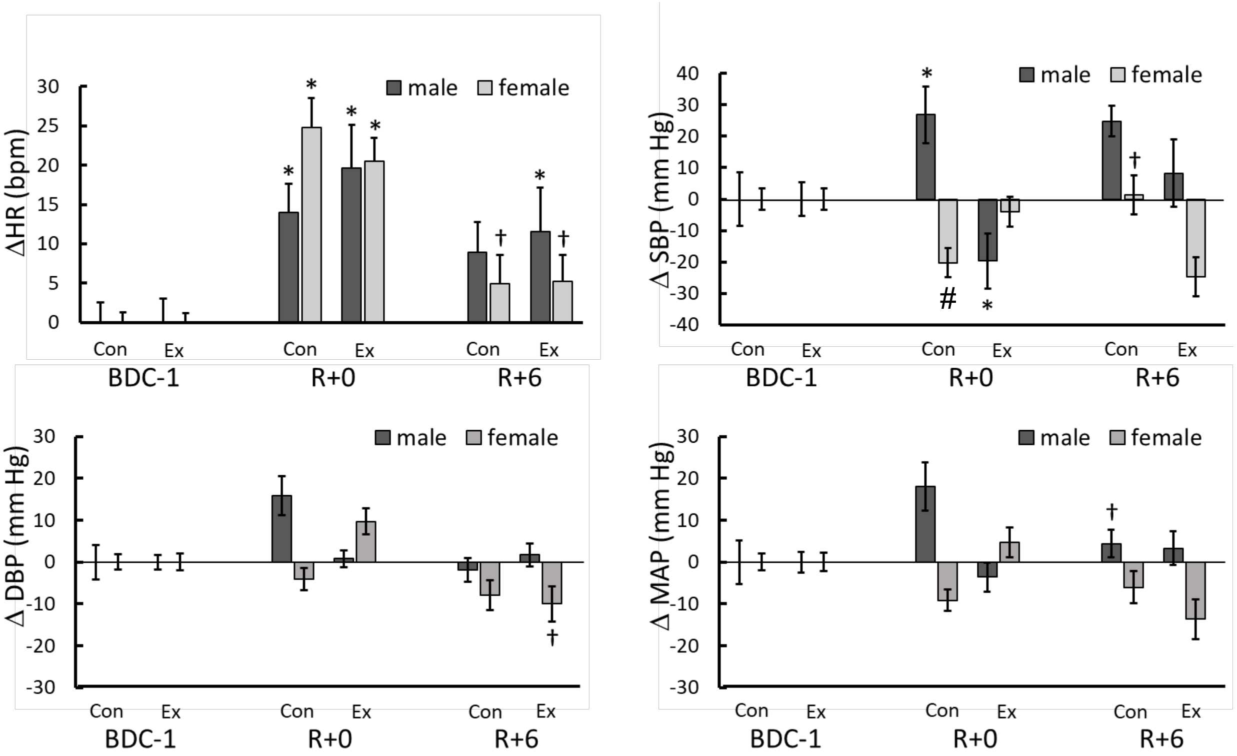


Figure 3.1. Heart rate and blood pressure change from BDC-1 (increase: positive; decrease: negative) for different sex and intervention groups on R+0 and R+6. *: significantly different from BDC-1, †: R+6 different from R+0. #: different from males in same day and intervention. ‡: the control and exercise groups were significantly different for the same sex.

Overall lower leg muscle activity was only significantly reduced with HDBR in the male control group (Table 3-2). 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 3.2).

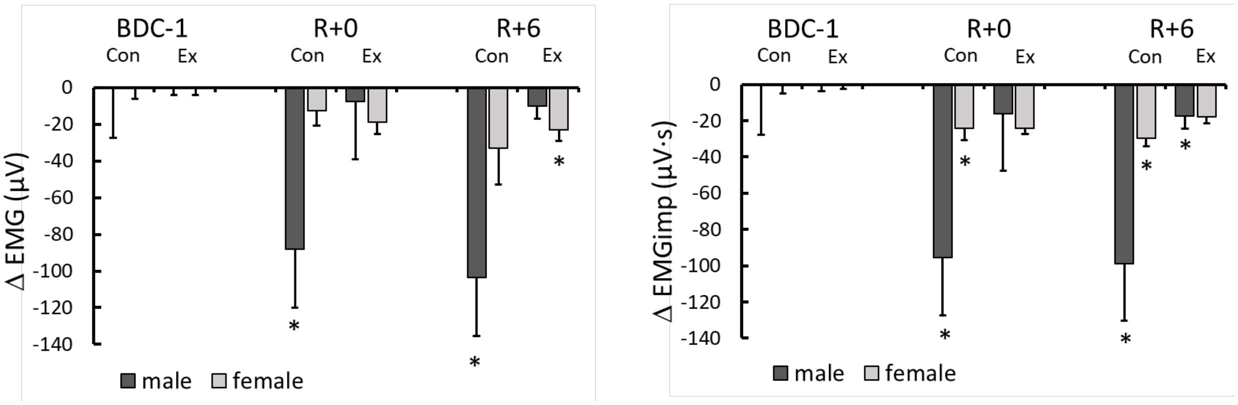


Figure 3.2. Electromyography (EMG) and electromygraphy impulse (EMGimp) changes from BDC-1 (increase: positive; decrease: negative) for different sex and intervention groups on R+0 and R+6. *: significantly different from BDC-1.

Table 3-2. 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 hours after the end of bedrest; R+6: six 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.

		Pre-bedrest (BDC -1)		**Post bedrest (R+0)**		**Post bedrest (R+6)**	
Variable	**Sex**	**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 $p < 0.05$.

3.4.3 Muscle-pump baroreflex

Following HDBR, the skeletal muscle-pump's ability to react to variations in BP was significantly reduced (Table 3-3). 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 3-3). 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.

Table 3-3. 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 hours after the end of bedrest; R+6: six 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		**Pre-bedrest (BDC -1)**		**Post bedrest (R+0)**		**Post bedrest (R+6)**	
	Sex	**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.

3.4.4 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 **3-4**). The fraction that the cardiac baroreflex was active (FTA) was significantly decreased in females only after 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 bedrest. 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 **3-4**).

Table 3-4. 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 hours after the end of bedrest; R+6: six 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		**Pre-bedrest (BDC -1)**		**Post bedrest (R+0)**		**Post bedrest (R+6)**	
	Sex	**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.

3.4.5 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 **3-3**). 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 (Table **3-4**). 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.

3.4.6 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 **3.3**). 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 **3.3**A). 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 **3.3**A).

The intervention and sex groups' responses in cardiac baroreflex functionality were more dramatic following HDBR compared to muscle-pump baroreflex outcomes (Figure **3.3**B).

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 **3.3**B).

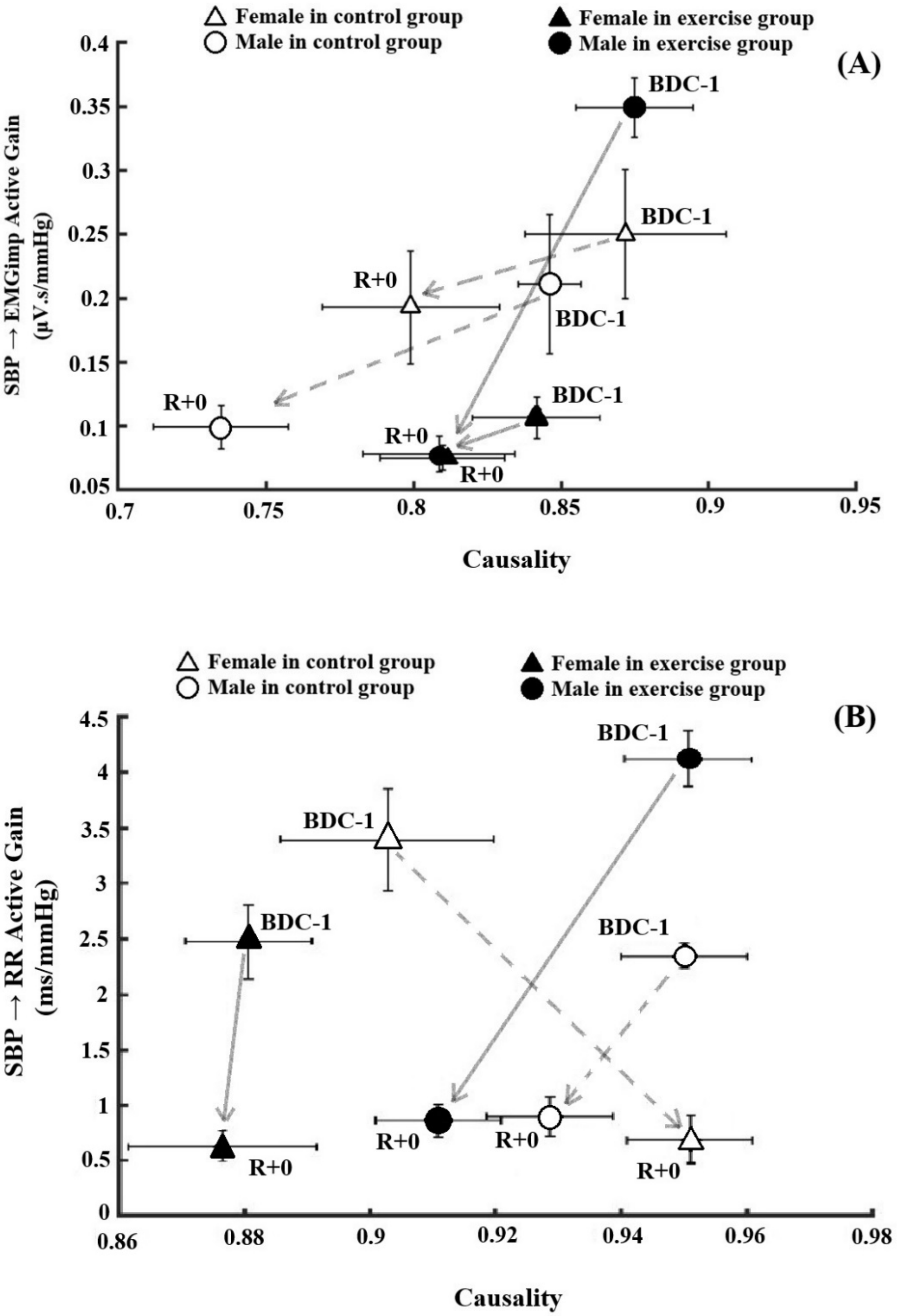


Figure 3.3. The association between causality and low frequency Active gain as a function of active interaction time (Active Gain: Gain X fraction time active) on pre bedrest (BDC-1) and R+0 related to (A) skeletal muscle-pump baroreflex system and (B) cardiac baroreflex system. The data in the circles 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.

3.5 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 of whom 60%, compared to 10% of male participants, were unable to complete six minutes of stand just hours after exiting from 14 days of bed rest. Given the drastic consequences on orthostatic tolerance, this paper is focused on two major components of the blood pressure control system, the cardiac and muscle-pump baroreflexes. Our results into orthostatic reflexes reveal that following two 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 [118] and younger [36] people after bedrest, simulated microgravity (e.g. HDBR) [82], or astronauts after spaceflight [116, 129, 133], respectively. Furthermore, the older composition of participants in this study adds to our understanding of the interaction of age with inactivity in the cardio-postural control system.

3.5.1 Muscle-pump baroreflex

We recently provided evidence of the importance of lower limb muscular contractions for the maintenance of standing blood pressure [36]. 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 [38, 91]. 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) [36]. 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 3-2)—most like related to the small sample sizes—changes from baseline were used to assess intergroup effects. EMGimp decreased significantly only in both the male and female control groups on R+0 and R+6 compared to pre-HDBR values (Figure 3.2). 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 3.2), indicating that they were better able to preserve muscle-pump capacity after lengthy periods of inactivity. 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 which indicated not only a probable drop in reflex output to the muscle but also a variation in activation [36]. 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 3-3). In fact, there was an across-the-board retention of muscle-pump baroreflex gain in all female participants post-bedrest. The male exercise groups on R+0 was not decreased significantly on R+0, 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 3-3).

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 [36]. 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 mechanical muscle-pump mechanics [36]. Finally, we examined the interaction between muscle-pump activity, the product of gain and FTA, with causality. Figure **3.3**A 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.

3.5.2 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 [134-136] and long-term [137] spaceflight. Bedrest has also been linked to decreased arterial baroreflex [3, 138-140].

We observed elevated standing HR following HDBR (Table **3-2**, Figure **3.1**), 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 increases commonly observed after bedrest as a compensatory reaction to increased venous pooling in the lower limbs through a lack of enhanced vasoconstriction [141-143]. 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 **3.1**). 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 [144]. 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 [144].

Our data revealed a considerable reduction in cardiac gain after HDBR (R+0) in all studied groups and in 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 the protective vascular effects of exercise in the male participants. Differences between the male and female participants suggest that along with vascular control, unique sex-related cardiac baroreflex control adaptations to exercise with bedrest deconditioning during standing.

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 or shorter in duration compared to pre-bedrest and to males. Furthermore, the female control participants had a significant increase in causality on R+0, whereas 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 **3.3**B). 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 3-2). 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 astronauts after spaceflight. Blaber et al. (Blaber et al., 2022) presented spaceflight data of equivalent duration (8-16 days) using similar analyses on 10-minute 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: a) weightlessness and HDBR are not equal in terms of unloading of the body since HDBR only removes the gravitational gradient from the head-foot axis of the body; b) the astronauts had a mean age was 39±5 year, 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 (Supplemental materials), may have had only months to prepare for HDBR; and 5) it 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.

3.5.3 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 [145] or to a period before a substantial change in hardware [145, 146], such as the replacement of iRED with ARED, is the only approach to assess the efficacy of the current exercise countermeasure in space. The restricted opportunity to conduct controlled intervention studies, both in space and in spaceflight analogs such as HDBR, is a substantial hurdle to developing a new exercise countermeasure [12, 147]. An exercise training intervention study is expensive and time-consuming in space. The 'SPRINT' research [148] 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 [149] and microgravity [150]. Even in this case, the control group was not fully deprived of physical activity and continued to perform routine ISS countermeasure exercises.

Terrestrial exercises (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. The improvement of ISS exercise countermeasure hardware (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 [151]. 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 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 tailored to each sex separately. 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 the baroreflex system, taking into consideration the sex-specific physiological effects.

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, and activation of the renin-angiotensin system and the sympathetic nervous system. [152-154]. In order to be eligible for participation in this study, women must be menopausal, as the majority of women aged 55-65 are menopausal and due to the fact that if we were to include both menopausal and non-menopausal women, we would be unable to accurately compare the women in the two groups [152, 154]. As a result, this study's findings focused on the response of older people to HDBR, making it difficult to draw comparisons with the outcomes of young females who are typically involved in bedrest studies.

3.5.4 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 [84] 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 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 of 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 (Fritch-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 **3-3**) 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 no-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 (Table **3-1**,Table **3-2**) 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 past through one-way valves. In this study we found that beat-to-beat EMG output to these muscles was reduced following bedrest (Table **3-2**). 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 in 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 the final resorts for a compromised cardiovascular, which 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 [155]. Astronauts, as we have examined earlier in this paper, are also susceptible to OH and have greater sway post-flight [156], while patients with autonomic failure often exhibit fidgeting leg behaviors when sitting [157].

3.6 Limitations and future works

The participants in this study were selected from a healthy older population aged 55 to 66 years old; however, many older people are on several medications and have substantial sarcopenia even before being placed on bedrest [118, 158]. 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 [118, 159, 160]. 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 [149, 161]; 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 in future studies. 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 [119], LBNP [162], pharmaceutical intervention [163], and artificial gravity [164] 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.

3.7 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.

Chapter 4. Cardio-Postural Interactions and Muscle-Pump Baroreflex of Individual Leg Muscles Following Bed rest in Older Adults

4.1 Abstract

Baroreflex-mediated activation of lower leg muscles plays an important role within the intricate dynamics of the blood pressure regulation system. Our previous research examined the efficacy of space-based exercises in preserving muscle-pump baroreflex function after 14 days of head-down tilt bed rest in 55-to 65-yr-old men and women. Yet, the impact of sex and exercise interventions on baroreflex activity on different lower leg muscles (tibialis anterior (TA), soleus (SOL), lateral gastrocnemius (LG), and medial gastrocnemius (MG)) during quiet standing remains unclear. Using data from the Canadian Aging and Inactivity Study, we analyzed the coherence and causality relationships between muscle activation and postural sway in regulating blood pressure during orthostatic challenges in older adults following bed rest. Employing Montecarlo simulation, wavelet transform coherence, and convergent cross-mapping, we explored the interdependence of blood pressure, postural sway, and lower leg muscles activation. This study presents significant sex-related adaptive strategies of the cardio-postural system for maintaining blood pressure upon standing. The investigation revealed a reduction in the muscle-pump fraction time active (FTA) of LG and TA muscles on the first recovery day (R+0) ($p < 0.03$) compared to the baseline before bed rest. There was a markedly higher muscle-pump gain in the MG as opposed to the TA and LG ($p = 0.001$ and $p = 0.02$, respectively) on R+0, showing a compensatory dependence on the MG during orthostatic challenges to enhance venous return. This was most prevalent in the male control group, where the greatest muscle electromyography (EMG) values for the MG were observed ($p = 0.001$) relative to other sex and intervention groups. In addition,

these data suggest that increases in postural sway in the female control group on R+0 ($p < 0.001$) resulted in a more pronounced interaction of the lower leg muscles with blood pressure (BP) during standing. This was further supported by the correlation of higher gain in the TA muscle EMG with blood pressure, along with increased FTA of sway with blood pressure and amplified causal relationship between sway and EMG on R+0 in female control participants' TA compared with the LG, MG, and SOL ($p < 0.001$, $p = 0.04$, and $p = 0.04$, respectively). These findings imply that the augmented postural sway in female control participants led to increased activation of the TA muscle, enhancing the muscle pump's functionality. Comprehending these alterations in postural and muscle-pump baroreflex related to physical activity or inactivity in older individuals is crucial for developing effective interventions to mitigate orthostatic intolerance in older male and female adults.

4.2 Introduction

In a gravitational environment, there is a shift of 500 - 1000 mL of blood from the central body compartments to the lower extremities and splanchnic circulation upon standing [11, 165]. This shift results in a decreased venous return to the heart and subsequently reduces cardiac output, leading to a decline in blood pressure (BP) and potential occurrences of syncope and falls. To counteract this orthostatic stress [166], several complex physiological interactions come into play. In healthy individuals, regulatory mechanisms swiftly compensate for the drop in blood pressure through baroreflex-mediated vagal withdrawal and sympathetic nerve activation to increase heart rate and systemic vascular resistance in response to a decrease in stroke volume [167]. The contraction of lower limb skeletal muscles facilitates the return of venous blood pooled in the legs back to the heart through the "muscle-pump," increasing venous return and blood pressure [38, 91].

Spaceflight-induced weightlessness triggers a cascade of multi-system changes [168], including reduced muscle size and strength, alterations in the cardiovascular system, decreased physiological interactions [169], and resetting of the carotid baroreceptors [108, 109]. These changes can impair reflex responses and result in a persistent drop in blood pressure upon returning to a gravity field, leading to visual disturbances, loss of consciousness, and increased risk of orthostatic intolerance (OI) and fainting, not only on the day of landing but also during subsequent recovery days [43].

Given the similarities between space adaptation and prolonged inactivity [170], physical exercise has been identified as a potential countermeasure to mitigate the effects of weightlessness-induced deconditioning [171]. Exercise equipment on the International Space Station (ISS) includes a cycle ergometer, treadmill, and the advanced resistive exercise device (ARED). These play a key role in preserving astronaut muscle and cardiovascular health [111]. Notably, the knowledge gained from space countermeasures could extend beyond astronaut health and provide valuable insights into age-related conditions and diseases on Earth [1, 109]. The complexity of age-related physiological changes, influenced by factors like hormones, exercise levels, diet, and illness, presents challenges in identifying the root causes of muscle loss and cardiovascular changes [115]. Studying the relationship between spaceflight exercises designed to counter similar alterations in musculoskeletal and cardiovascular systems with aging can offer novel perspectives and innovative techniques to incorporate into Earth-based medicine and rehabilitation [1, 109, 116].

Because of the limitations of conducting human research in space [85, 86], head-down tilt bed rest (HDBR) serves as a valuable terrestrial platform for simulating microgravity exposure and studying the effects of weightlessness on the human body [172]. Physiological changes observed during and following HDBR share similarities with those observed in spaceflight, including disruptions in the cardiovascular system [173, 174] and postural stability [175], which has assisted in developing comprehensive countermeasures against microgravity-induced deconditioning [118, 119]. However, despite decades of research, a fully effective countermeasure against space-induced cardio-postural deconditioning has not yet been developed [93].

It is noteworthy that participants in HDBR studies have a median age of only 24.5 years [23], whereas astronauts are typically older, with an average age of 40.9 before their first flight and 45.3 after their last flight (excluding John Glenn, who was 77.3 at the time of his last flight) [24]. Only a few HDBR studies involving older adults have incorporated exercise countermeasures, with participants maintaining a head-down tilt position [25]. Considering these knowledge gaps and the increase in the global elderly population [107], the current study aimed to investigate the effects of HDBR and inactivity on older adults.

Previously [34], we quantified the efficacy of space-based exercise countermeasures on the inter-relationship between cardiovascular and postural systems with participants in the Canadian Aging and Inactivity Study (CAIS). Lower limb reflex-mediated muscle responses to blood pressure changes (muscle-pump baroreflex [37, 53, 176]) were both sex and exercise-intervention-dependent [34]. This implied that individualized countermeasures may be necessary [177-180]. To tailor exercises, we must first understand the contribution of individual muscles to the muscle-pump baroreflex and the effect of HDBR on those responses [93]. Such knowledge could clarify the role of lower limb inactivity in the input of individual muscles to blood pressure regulation. In this analysis of CAIS data, we investigated the individual roles of the lateral gastrocnemius (LG), medial gastrocnemius (MG), tibialis anterior (TA), and soleus (SOL) in the muscle pump baroreflex towards facilitating venous return to the heart in older men and women. We studied the effectiveness of combined lower body strength, aerobic, and high-intensity interval training (HIIT) exercises on the function of muscle pump baroreflex, postural sway, and blood pressure regulation following 14 days of HDBR [84].

4.3 Materials and Methods

4.3.1 Study Design and Testing Protocols

The Canadian Space Agency (CSA) in partnership with the Canadian Institutes of Health Research (CIHR) and the Canadian Frailty Network (CFN), supported research into a new space-inspired exercise program to preserve the cardiovascular and musculoskeletal systems from deconditioning following 14 days of HDBR [84]. Participants underwent a 26-day HDBR campaign. They checked into the facility five days for baseline data collection (BDC) and acclimated to the new environment before being placed in six degrees of continuous downward inclination bed rest using a pillow for 14 days. This was then followed by seven days of recovery (R) for post-HDBR measures and to ensure sufficient rehabilitation before leaving. During the HDBR, half of the participants partook in prescribed exercise countermeasures (exercise group [84]), while the other half served as controls and received standard stretches and joint movement physiotherapy (control group).

The exercise group engaged in 60 to 75 minutes of daily physical activity, which comprised a combination of three exercise sessions from high-intensity interval training (HIIT), low-intensity aerobic activity, and lower body strength exercises. A detailed description of the exercise protocols is provided by Hedge et al. along with the rationale for their implementation [84]. Briefly, cycling and resistance training with resistive bands were prescribed for muscular and cardiovascular health, resembling a high intensity, low-volume program [84]. HIIT was added to the exercise program to improve baroreflex responses, aerobic fitness, and cardiovascular health [84]. All exercise sessions were performed in the six-degree head-down tilt posture, while the intensity was adjusted based on the individual's heart rate, blood pressure, performance, and tolerance. Standards of care provided to either group were identical, except for the exercise sessions.

The study was reviewed and approved by the MHUC research ethics board and registered in the US National clinical trial registry (clinicaltrails.gov NCT04964999: Microgravity Research Analogue: Understanding the Health Impact of Inactivity for the Benefit of Older Adults and Astronauts Initiative). The Office of Research Ethics at Simon Fraser University gave ethics approval to conduct the research and collect the data for our portion of the study. Participants signed an informed consent form and committed to spending all 26 study days at MHUC. All research was conducted in accordance with the Declaration of Helsinki.

4.3.2 Participants

Of the 219 candidates who expressed an interest in the study, twenty-three participants were enrolled after being screened using inclusion and exclusion criteria (Supplemental material). Following random assignment by sex into exercise and control, there were four test groups, including male and female, in the control and exercise groups by the MUHC staff. Of the twenty-three healthy men and women aged 55 to 65, two were excluded from our analyses because of medical issues, and one for poor data quality.

4.3.3 Data Collection

Using the Bagnoli-8 (Delsys Inc, MA, USA) surface electromyography (EMG) system, transdermal recordings from four bilateral lower leg muscles were collected: the tibialis anterior, lateral soleus, and medial and lateral gastrocnemius. Bipolar surface EMG recordings may be affected by the shape, size, position, and orientation of the electrode, as well as inter-electrode distance. The EMG sensors are equipped with internal shielding to block external electrical noise. The contacts are constructed from 99.9% pure silver bars, each 10mm in length and 1mm in diameter, spaced 10mm apart to ensure optimal signal capture and consistency. The enclosure's curved design enhances skin contact and adhesion while reducing the negative impact of sweat during exercising. To achieve maximum signal detection, sensors were positioned parallel to the muscle's fiber orientation and placed in the center of the muscle belly, away from tendons and muscle edges. The sensor is easily attached to the skin using the Delsys Adhesive Sensor Interface, which facilitates a high-quality electrical connection between the sensor bars and the skin, minimizing motion artifacts and line interference. For a secure attachment, remove excess hair and clean the skin and EMG sensor with alcohol to eliminate oils and surface residues, allowing the skin to dry completely before applying the interface.

Cables were secured using tape and elastic fabric wrap to reduce the risk of movement artifacts caused by destabilization of the electric layer and changes in impedances and magnetically induced currents in the cables. To ensure the reliability of EMG signals recorded across different test days, despite variability due to factors like subcutaneous tissue thickness and electrode-skin impedance, several methodological controls were applied. Electrode placement was standardized using anatomical landmarks and positions recommended by the SENIAM project (Surface Electromyography for the Non-Invasive Assessment of Muscles) [87]. Repeatability and reliability were assessed by comparing test results from five days (familiarization test) and one day (first StS test) before bed rest. A regular Student's t-test with a Bonferroni adjustment of the mean value of filtered, rectified, normalized and enveloped EMG signals indicated no significant differences within participants and between groups on these days. These procedures ensured that differences in EMG signals, pre- and post- bedrest, were due to physiological changes rather than measurement artifacts.

Electrocardiograms (ECG) were captured using a bipolar three-lead ECG (IX-BIO4, iWorx, USA) with the electrodes arranged in a standard Lead III configuration. Blood pressure (BP) was continuously monitored at the finger using the non-invasive Portapres (FMS, Amsterdam, The Netherlands), with absolute BP height-corrected to the level of the heart. The National Instruments USB-6218 16-bit data capture hardware and LabVIEW 2013 software were used to record the data at a rate of 1000 Hz (National Instruments Inc, TX, USA).

4.3.4 Supine-to-Stand Test Procedure

To activate and evaluate the cardio-postural control system [37, 53, 88-91], a supine-to-stand (StS) test was performed twice during BDC (on BDC-5, and BDC-1) and twice on recovery days (on R+0, R+6) [34]. The first BDC-5 StS test was considered a familiarization protocol [34]. Note that the StS tests on BDC-1 and R+0 occurred an hour after the standard tilt test mandated by the CSA, which lasted up to 15 minutes [85, 86]. Participants were requested to lie supine on a bed in the testing room while being instrumented for physiological monitoring. The lights were then turned off, and the subjects were advised to close their eyes for five minutes during supine data acquisition. After this baseline, participants were assisted to stand on the force plate where they remained for six minutes with their feet 5 cm apart, eyes closed, and arms at their sides while the stand data was recorded. Sadeghian et al. have previously comprehensively explained the StS test [34].

4.3.5 Data Analysis

We followed the data analysis steps outlined by Xu et al. [53]. In brief, RR intervals were determined with the help of the ECG signal. Beat-to-beat SBP and diastolic blood pressure (DBP) were calculated from the highest and lowest points in the BP waveform during a heartbeat. The mean BP from end-diastole to end-diastole of the waveform was used to calculate mean arterial pressure (MAP).

Methodologies for analyzing EMG vary significantly among scientific groups. This lack of consistency hinders the widespread application of bed rest studies, making standardization crucial for analyzing EMG signals. In this study, we follow the recommendations of the SENIAM project to address key issues that prevent effective data exchange and clinical experience sharing. The SENIAM project united the expertise of 16 European groups specializing in the development and application of surface EMG.

Band-pass filtering was applied to reduce noise and artifacts. The selection of a high-pass filter on the low-frequency side of the EMG signal spectrum was primarily driven by the need to eliminate slow variations caused by movement artifacts. Typically, a high-pass filter set between 10 and 20 Hz will retain the critical frequencies in the surface EMG signal. However, the 5-20 Hz frequency range also contains information about the firing rates of active motor units. It's important to note that sudden signal changes due to movements may not be fully attenuated by a 5-20 Hz filter. Since we were interested in cardio-postural interactions occurring below 0.5 Hz, we chose a cut-off frequency of 5 Hz. On the high-frequency side, approximately 95% of the surface EMG power is captured by harmonics up to 400 Hz, with most remaining power attributed to electrode and equipment noise. To further attenuate these unwanted components, a low-pass filter with a cut-off frequency set to 400 Hz was applied to the signal. Given our sampling frequency was 1000 samples/second, following the sampling theorem, which requires sampling at more than twice the highest frequency in the signal.

The purpose of this study was to explore the role of individual lower leg muscles in the muscle pump baroreflex system. To preserve the integrity of our comparisons across muscles, we have not normalized the data based on muscle mass. Normalizing in this manner could obscure inherent differences between muscle groups, reducing the effectiveness of our analysis in distinguishing their individual contributions to the baroreflex system. However, many other factors affect EMG signals, and it is challenging to describe muscle voltage levels without a reference value for comparison or a normalization procedure is applied.

The EMG signals were normalized based on peak activation levels during maximum contractions, allowing for comparison of EMG activity within the same muscle across different days, between different individuals, or between different muscles. These signals were then rectified and smoothed to minimize noise and artifacts. The area under the rectified EMG envelope between successive heartbeats was used to calculate the beat-to-beat EMG impulse (EMGimp: $\mu v.s^{-1}$) of each lower leg muscle. Four groups representing the bilateral values for the medial gastrocnemius (EMG-MG), lateral gastrocnemius (EMG-LG), tibialis anterior (EMG-TA), and soleus (EMG-SOL) were created from the rectified EMG recorded from four different muscles in each leg. The beat-to-beat physiological signals were re-sampled to 10 Hz using a spline-based interpolation method before wavelet transform coherence and causality studies had been conducted.

Time-frequency distributions of each signal pair of SBP with EMGimp for each muscle (MG, LG, TA, and SOL), and mean postural sway (COPr), anterior-posterior sway (COP_{AP}), and medio-lateral sway (COP_{ML}) were generated using a Morlet wavelet [37, 89]. To ensure statistical robustness, 1,000 pairs of surrogate data were generated for each pair of signals analyzed. These surrogate data were generated using a first-order autoregressive process model, with coefficients estimated from the actual signals. Subsequently, wavelet coherence was computed for all pairs of surrogate data, and the coherence threshold was set at the 90th percentile of the coherence sampling distribution for each scale/frequency, as established using the Monte Carlo method [53]. The Monte Carlo's threshold is essential for accurately identifying and characterizing the correlation between each pair of signals. Only the low-frequency band (LF, 0.07-0.15 Hz) was studied in this research as it has been shown that this frequency band is associated with cardio-postural coupling and the muscle-pump baroreflex [53].

The fraction of total time when there was active interaction was calculated by dividing the area above the significant coherence threshold in the low frequency band by the total area in this band (Fraction Time Active: FTA). For each of the above signal pairs, the value of the response gain was calculated by averaging the cross wavelet transform of the signals over the most significant regions in the low frequency range [102]. Then, 'Active Gain', (Gain × FTA) was introduced as a supplementary method for describing the efficacy of each interaction [36]. The signal pair's causal relationship was determined using the convergent cross-mapping method [105]. Supplemental information regarding the procedures can be found in Sugihara et al. [105].

To better understand the interrelationships between baroreflex- and postural-related muscle contractions in the low-frequency band across all groups under study, we examined the pairwise interactions between the involved systems. We specifically looked at: (1) how muscle activity interacts with postural sway (EMG↔COPr), (2) how postural sway interacts with blood pressure (COPr↔SBP), and (3) how the electrical activity of muscles interacts with blood pressure (EMG↔SBP). The percentages of significant coherence (%SC) in the low frequency band for each paired signal were measured using the approach proposed and comprehensively explained by

Torrence and Compo [101]. This frequency range (0.07–0.15 Hz) was chosen because it reflects the reaction to disturbances in the cardiovascular and postural systems. These results are visualized using Venn diagrams containing three ellipses, representing the FTA between signal pairs [i.e., EMG↔COPr, EMG↔SBP, and COPr↔SBP]. The overlapping sections indicate where two or more pairs of signals are simultaneously coupled, working together to achieve a coordinated approach.

4.3.6 Statistical Analysis

The interquartile range method was used to delete outliers and ensure that all cardio-postural values and interrelationship factors were meaningful in the statistical analysis. Data were considered an outlier if the value was over 1.5 times the interquartile range, larger than the third quartile, or less than the first quartile. Each of the four groups of participants had their separate application of the *winsorization* approach for handling outliers [181]. Responses on BDC-1 were used as a baseline to determine differences due to the small sample size per group. After comparing values within each group to their respective pre-HDBR values, we compared the groups' responses as changes from BDC-1. Increases were interpreted as positive and decreases as negative.

Since not all response variables were normally distributed, we used a nonparametric ANOVA-type statistic (nparLD, F2-LD-F1 design) suggested by Brunner et al. [131] to examine the effect of sex (male, female), intervention (control, exercise), and test days (BDC-1, R+0, and R+6), as well as their interactions, on the response variables. The F2-LD-F1 is an experimental design that includes two between-subjects factors (sex and intervention) and one within-subjects factor (test days). We used multiple comparisons (LD-F1 design) with Bonferroni adjustment to study the pairwise differences between BDC-1, R+0, and R+6 (time main effect). To investigate the differences between male controls, female controls, male exercise, and female exercise (treatment main effects) at BDC-1, R+0, and R+6, the Kruskal-Wallis test was performed, followed by the Conover-Iman post-hoc test. Similarly, we used Kruskal-Wallis and Conover-Iman post-hoc tests for multiple comparisons between the individual lower limb muscles (MG, LG, TA, and SOL). All statistical tests were performed using R [132], and data are reported as significant ($p<0.05$) or trends ($0.1>p\geq0.05$).

4.4 Results

In this study, the results of the stand portion of the StS tests are reported. The minute of data associated with the transition from supine to stand was not used since it contained motion-related disturbances and with our focus on the interaction between muscle activity and blood pressure regulation within the muscle pump baroreflex system, we required steady-state conditions rather than positional changes in muscle activity. After one minute, when the participant's feet were properly positioned, their body facing forward, and they could stand without assistance, the stand clock was started, and the first 180 seconds of the stand were analyzed.

4.4.1 Postural and Musculoskeletal Responses

To validate our EMG measurement for all lower leg muscles and ensure high-quality data recording in line with SENIAM project recommendations, we compared EMG signals from BDC-5 and BDC-1. This comparison assessed the variability in the recorded data. A Student's t-test with Bonferroni adjustment comparing the average EMG signals across two days for all participants while standing indicated no significant differences ($p > 0.27$).

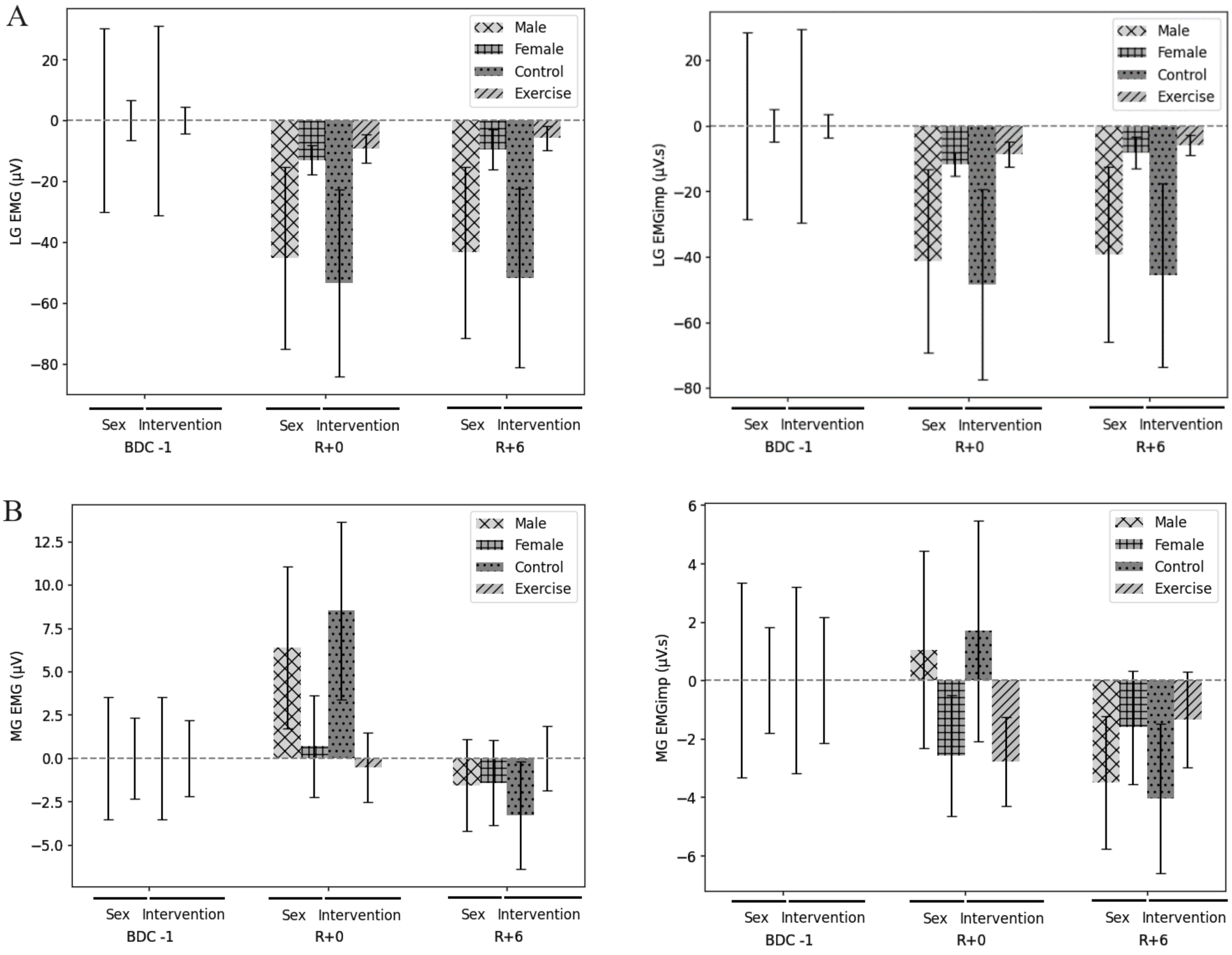
Sex or intervention dependent responses to the consequences of the HDBR were observed in different muscle groups (Figure **4.1**). Reductions in EMG activity was predominantly observed in the LG and TA muscles ($p < 0.001$), whereas the MG and SOL muscles did not exhibit significant decreases in activity on the first recovery day (R+0) compared to baseline measurements ($p > 0.20$) (Table **4-1**). This decline in EMG activity was specifically noted in male participants ($p < 0.01$), and it did not recover even after six days of recovery on R+6 StS test ($p < 0.01$). Female participants did not show significant changes ($p > 0.30$). There was a significant reduction in the LG EMG activity in the control group ($p = 0.03$), but not in the exercise group.

Sex-dependent responses in beat-to-beat EMG impulse, EMGimp, mirrored the patterns observed in overall EMG activity. Our results indicated a significant reduction in LG and TA EMGimp in male participants on R+0 ($p < 0.01$), which persisted until R+6, whereas no significant changes were observed in female participants ($p > 0.50$). Additionally, the SOL muscle also showed a significant reduction in EMGimp on R+0 in both male and female participants. The significant reduction in EMGimp of all lower leg muscles except MG was only observed in control participants ($p < 0.01$), but not in the exercise group ($p > 0.54$) (Table **4-1**).

Table 4-1. Mean (± standard error) standing muscle activity values for different groups including males, females, control and exercise groups on BDC -1, R+0 and R+6. Mean muscle activity values were obtained from the stand phase of the supine-to-stand test. BDC -1: baseline data collection day −1; R+0: 2 hours after the end of bed rest; R+6: six days after bed rest; EMG: electromyogram; EMGimp: Electromyogram beat-to-beat impulse; LG: Lateral gastrocnemius; MG: Medial gastrocnemius; TA: Tibialis anterior; SOL: Soleus.

Variable	Sex	BDC -1	R+0	R+6	intervention	BDC -1	R+0	R+6
LG EMG (µV)	**Male**	61.91±1.52	16.68± 1.23*#	18.48± 1.53*#	**Exercise**	21.62±5.97	12.68±3.31‡	15.72±3.41‡
	Female	27.95 ± 4.77	14.8 ± 2.74#	17.21± 2.32#	**Control**	68.25±7.52	18.81±3.73*‡	19.98±4.05‡
LG EMGimp (µV·s)	**Male**	53.18 ± 5.01	11.83± 2.47*#	13.86± 1.73*#	**Exercise**	17.32±5.11	8.71±3.58‡	11.22±2.52‡
	Female	21.39 ± 3.31	9.17 ± 2.03*#	12.18± 2.08#	**Control**	57.26±7.35	12.29±2.90*‡	14.83±3.27*‡
MG EMG (µV)	**Male**	22.59 ± 2.99	28.99 ± 4.93	21.04± 2.10	**Exercise**	17.23±2.98	16.50±3.89	17.06±1.97
	Female	18.27 ± 2.12	18.78 ± 2.80	16.14± 1.63	**Control**	23.64±4.64	31.26±7.23	20.12±3.43†
MG EMGimp (µV·s)	**Male**	19.28 ± 2.99	20.32 ± 2.91	15.78± 1.99	**Exercise**	14.11±2.99	11.18±3.61	12.55±2.25
	Female	14.13 ± 1.59	11.24 ± 1.95	11.92± 1.48	**Control**	19.31±4.22	20.38±3.25	15.14±2.60
TA EMG (µV)	**Male**	26.21 ± 4.51	12±2.37*#γ	13.22± 2.10*	**Exercise**	16.83±3.09	20.38±4.09	10.32±2.10
	Female	15.58 ± 1.94	22.87 ± 2.89#	10.01± 1.06†	**Control**	24.96±6.47	15.30±3.51	12.91±2.75
TA EMGimp (µV·s)	**Male**	22.50 ± 3.81	8.53± 1.45*#δ	10.02± 1.82*	**Exercise**	12.96±1.92	11.91±3.36	7.51±1.68
	Female	11.81 ± 1.41	13.10 ± 2.74#	7.10 ± 0.67†	**Control**	21.35±6.31	9.72±2.41*	9.60±2.22
SOL EMG (µV)	**Male**	40.17 ± 4.52	26.63 ± 3.09γ	26.22± 2.16	**Exercise**	25.93±5.93	20.34±4.45	23.37±5.55
	Female	28.16 ± 3.45	17.74 ± 1.85	18.50± 2.70	**Control**	42.40±6.23	24.04±3.27	21.35±3.13
SOL EMGimp (µV·s)	**Male**	34.26 ± 4.83	18.82± 3.25*δ	18.81± 1.96*	**Exercise**	21.02±6.86	13.92±4.07	16.82±3.78
	Female	21.43 ± 3.26	10.82 ± 1.38*	13.46± 2.00	**Control**	34.67±6.02	15.72±2.86*	15.44±2.20

Legend: *: significantly different from BDC-1, †: R+6 different from R+0, #: 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. Greek symbols: Values with similar Greek symbols were significantly different between the corresponding leg muscles in the same variable in the same group on each day. Significance was set at $p < 0.05$.


A
Male
Female
Control
Exercise
20
0
−20
−40
−60
−80
LG EMG (µV)
Sex
Intervention
BDC -1
R+0
R+6
LG EMGimp (µV.s)
B
12.5
10.0
7.5
5.0
2.5
0.0
−2.5
−5.0
MG EMG (µV)
6
4
2
−2
−4
−6
MG EMGimp (µV.s)

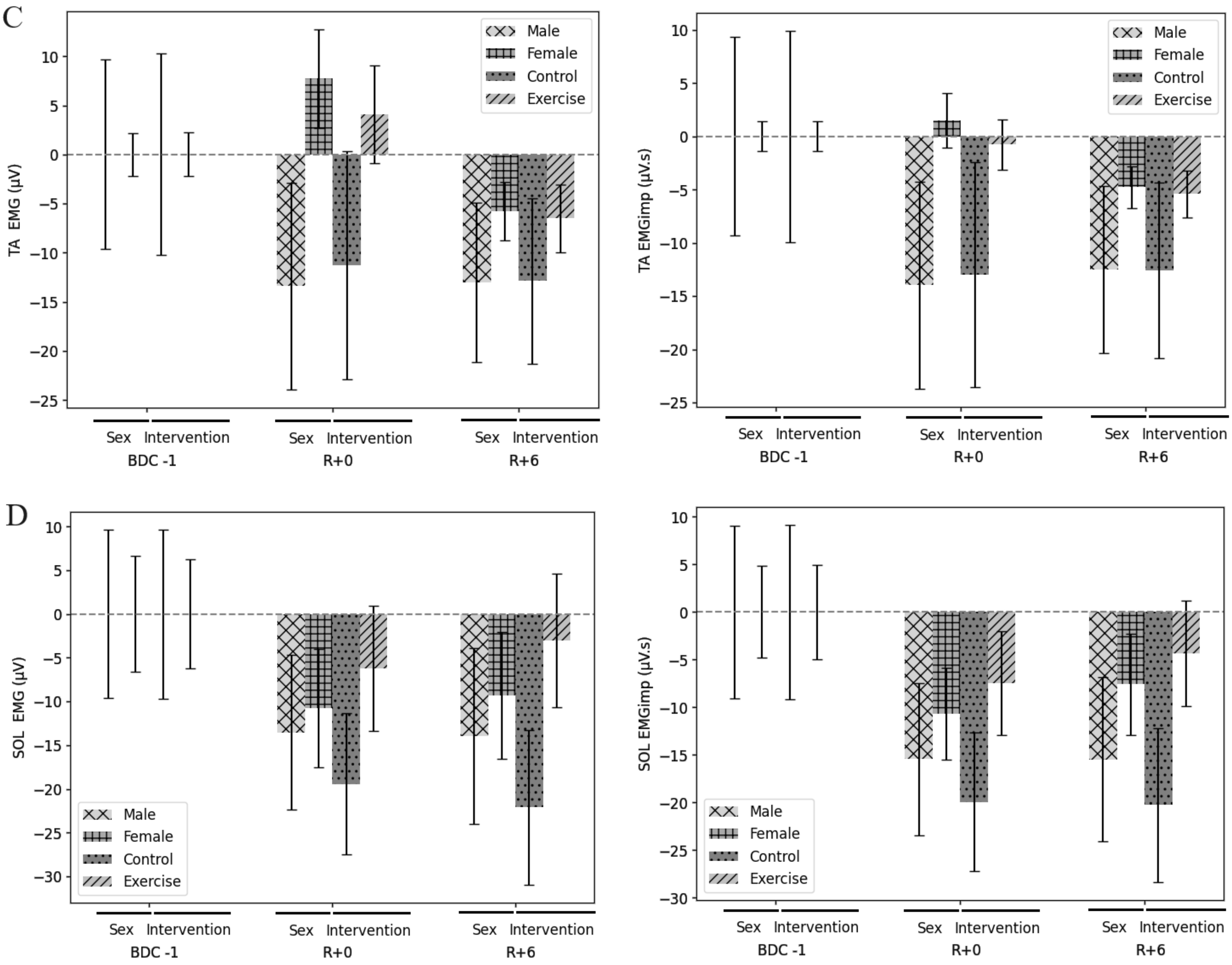


Figure 4.1. Changes from BDC-1 (increase: positive; decrease: negative) in muscle activity (EMG) and beat-to-beat muscle activity (EMGimp) of different studied lower leg muscle groups for different sex and intervention groups across different test days. A: lateral gastrocnemius, B: medial gastrocnemius, C: tibialis anterior, D: soleus

The effects of 14 days of HDBR on postural sway variables were significant (Figure **4.2**). During quiet stand on R+0, 2 hours after HDBR, a significant increase from the BDC-1 baseline in the average resultant postural sway (COPr) was observed with all participants ($p < 0.001$), regardless of sex or intervention (Table 4-2 and, Figure 4.2). Significant reversals in COPr responses from R+0 were observed on R+6 in all participants ($p < 0.01$). Additionally, both females and the control group participants showed significant increases in postural sway velocity (COPrv) responses on R+0 compared with baseline values ($p < 0.001$), which significantly recovered on R+6 from R+0.

Table 4-2. Mean (± standard error) standing postural sway values for different groups including males, females, control and exercise groups on BDC -1 and R+0 and R+6. Mean postural sway values were obtained from the stand phase of the supine-to-stand test. BDC -1: baseline data collection day −1; R+0: 2 hours after the end of bed rest; R+6: six days after bed rest; COP: Displacement of center of pressure; COP_v: Velocity of movement of COP; r: Resultant sway; AP: Anterior-posterior sway; ML: Medial-lateral sway.

Variable	Day Sex	BDC -1	R+0	R+6	Day intervention	BDC -1	R+0	R+6
COPR (mm)	Male	4.99 ± 1.06	7.97± 1.21*#	5.32 ± 0.38	Exercise	4.54±0.89	8.92±1.74*	5.14±0.52
	Female	4.69 ± 0.29	9.96± 1.42*#	5.19 ± 0.52	Control	5.15±1.45	9.01±2.52*	5.37±0.84
COPR_v (mm/s)	Male	11.10± 1.48	20.88± 3.07*#	13.95± 1.60	Exercise	11.54±2.41	20.36±1.63	13.35±1.54
	Female	11.50± 1.42	29.99± 2.96*#	12.55± 1.51†	Control	11.05±1.73	30.50±6.47*	13.15±2.96†

Legend: *: significantly different from BDC-1, †: R+6 different from R+0, #: 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 $p < 0.05$.

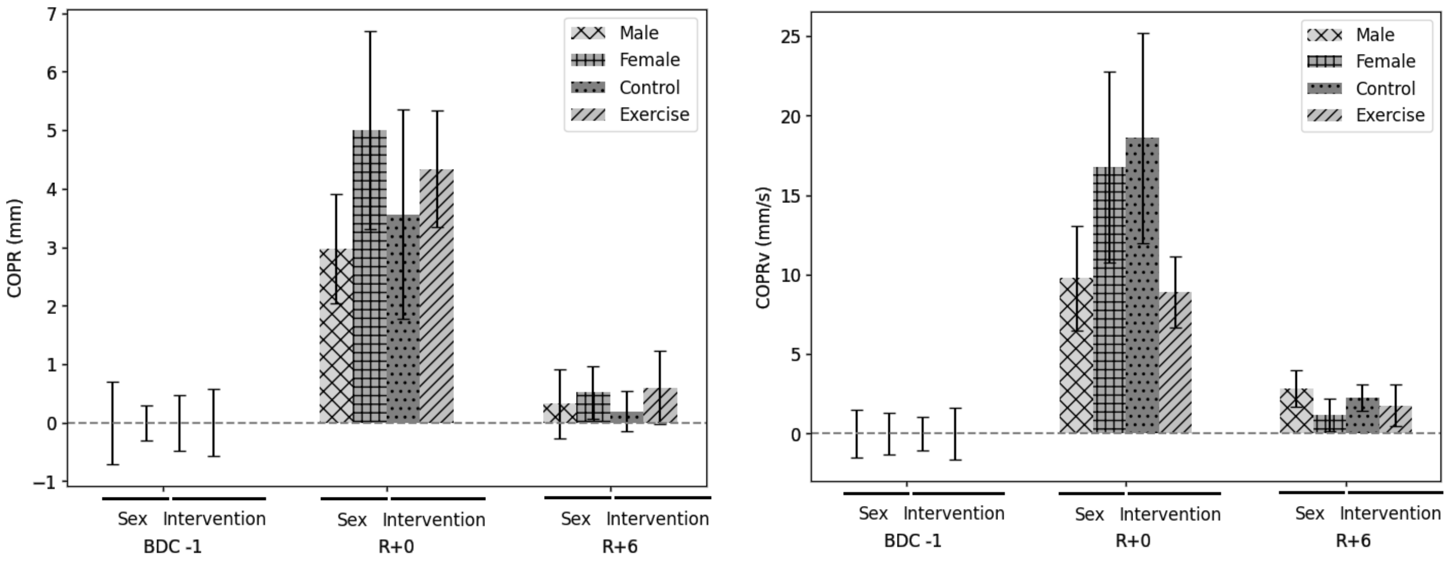
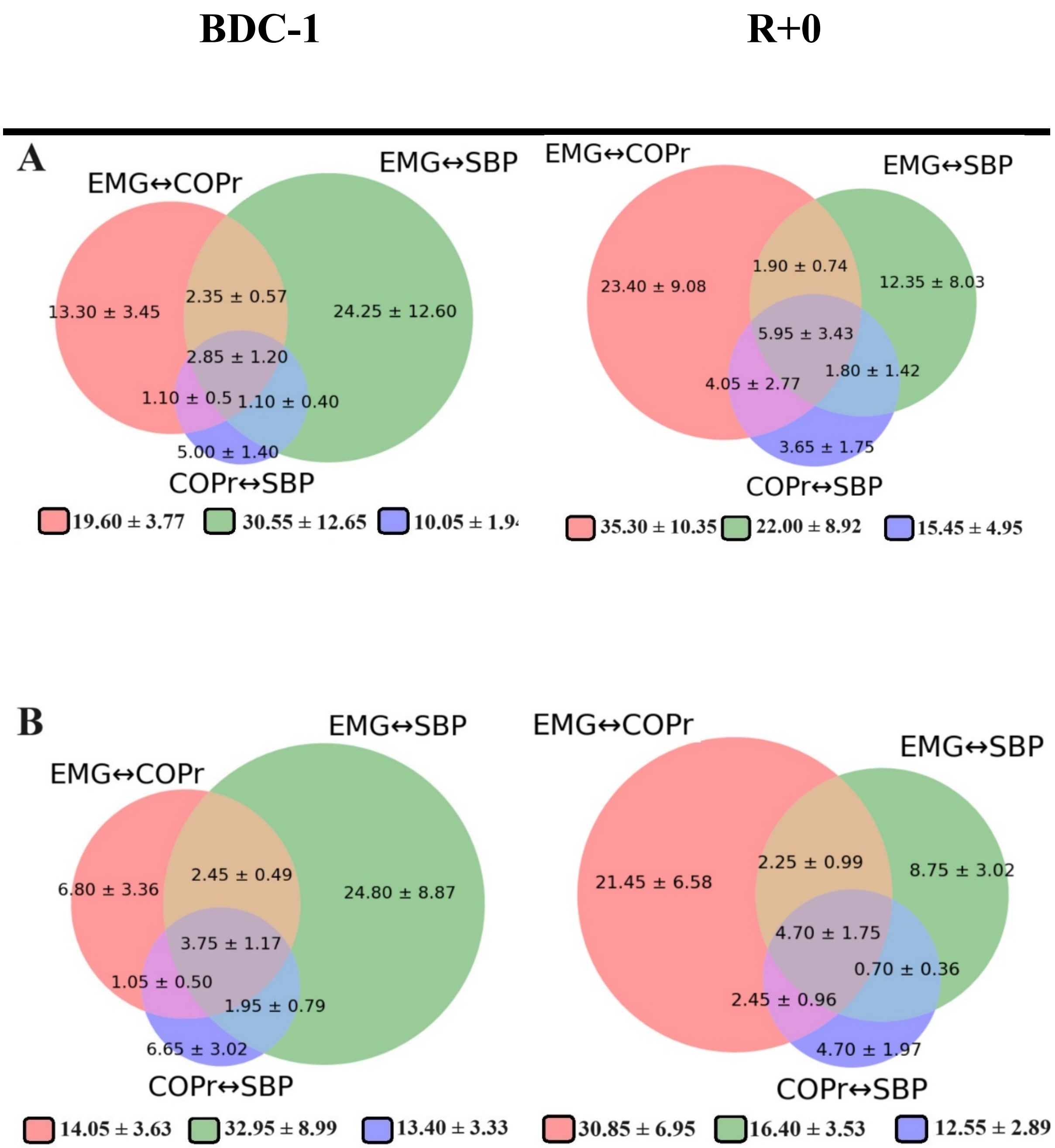


Figure 4.2. Changes from BDC-1 (increase: positive; decrease: negative) in center of pressure (COP) and mean velocity of movement of COP (COPv) of resultant CoP (COPr) for different sex and intervention groups across different test days.

4.4.2 Cardio-Postural Interactions

To differentiate between baroreflex- and postural-mediated muscle contractions, BP (SBP), sum EMG from individual muscles, and postural sway (COPr) signal pairs were analyzed as EMG↔COPr, EMG↔SBP, and COPr↔SBP. These results were visualized using Venn diagrams (Figure **4.3**). There was an almost doubling of postural sway, represented by the red zone, in these participants. When examining these data in terms of interactions, we found a significant increase ($p < 0.001$) on R+0 across all investigated groups compared with their baseline values, with many cases showing a doubling effect.

The significant interaction between muscle activities and SBP, denoted in green, exhibited a substantial decrease in all groups on R+0 compared with their corresponding BDC-1 values ($p < 0.001$), with no interaction between sex and interventions for the observed changes. The third zone under consideration was the center of pressure with blood pressure. When posture changes, it inevitably affects blood pressure. On R+0, females and the control group showed a significant change in the region exclusive to blood pressure interactions with postural sway (SBP↔COPrv, blue color) compared with the pre-HDBR responses ($p < 0.01$), but this was not observed in other groups ($p > 0.13$). Examining the central overlap component between all systems revealed a significant increase in both groups ($p < 0.001$), but not in males and the exercise group. Furthermore, the overlapped regions where posture-related blood pressure interactions (SBP↔COPrv) intersect with both green and red areas were significantly higher in females on R+0 compared with their baseline ($p = 0.007$), unlike other groups ($p > 0.1$).


BDC-1
R+0
A
EMG↔COPr
EMG↔SBP
13.30 ± 3.45
2.35 ± 0.57
24.25 ± 12.60
2.85 ± 1.20
1.10 ± 0.5
1.10 ± 0.40
5.00 ± 1.40
COPr↔SBP
🟥 19.60 ± 3.77
🟩 30.55 ± 12.65
🟦 10.05 ± 1.9
EMG↔COPr
EMG↔SBP
23.40 ± 9.08
1.90 ± 0.74
12.35 ± 8.03
5.95 ± 3.43
4.05 ± 2.77
1.80 ± 1.42
3.65 ± 1.75
COPr↔SBP
🟥 35.30 ± 10.35
🟩 22.00 ± 8.92
🟦 15.45 ± 4.95
B
EMG↔SBP
EMG↔COPr
6.80 ± 3.36
2.45 ± 0.49
24.80 ± 8.87
3.75 ± 1.17
1.05 ± 0.50
1.95 ± 0.79
6.65 ± 3.02
COPr↔SBP
🟥 14.05 ± 3.63
🟩 32.95 ± 8.99
🟦 13.40 ± 3.33
EMG↔COPr
EMG↔SBP
21.45 ± 6.58
2.25 ± 0.99
8.75 ± 3.02
4.70 ± 1.75
0.70 ± 0.36
2.45 ± 0.96
4.70 ± 1.97
COPr↔SBP
🟥 30.85 ± 6.95
🟩 16.40 ± 3.53
🟦 12.55 ± 2.89

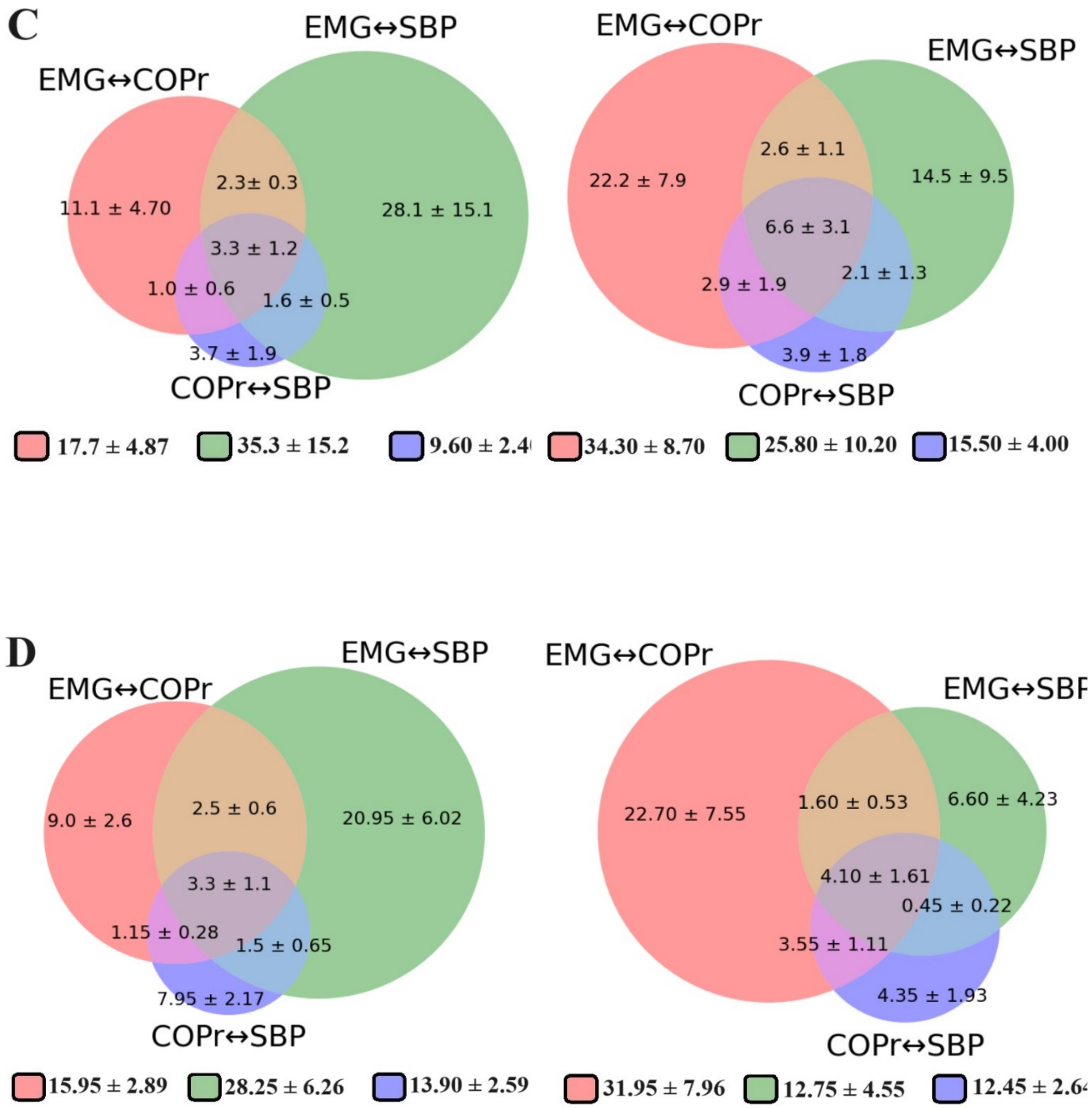


Figure 4.3. Significant coherence (%SC) in the low frequency range between different signal pairs was found using wavelet coherence between the cardiovascular and postural control systems. Three ellipses make up each Venn diagram, and their combined area represents the total number of interactions between any given pair of signals [i.e., EMG↔COPr, EMG↔SBP, and COPr↔SBP]. The overlapping areas denote simultaneous coupling of multiple pairs of signals. A: Females, B: Males, C: Control group, D: Exercise group.

4.4.3 Individual Muscles' Contribution to Muscle-Pump Baroreflex

There were significant differences in the muscle-pump baroreflex fraction time active (FTA) response between the intervention and sex groups across the different test days and muscle groups (Table 4-3 and Table 4-4). On R+0, the LG was the only muscle group to show a significant decrease in FTA response across all sex and intervention groups ($p < 0.01$). Only males failed to recover by R+6 in LG FTA ($p = 0.012$). Additionally, the FTA of the males and exercise groups for the TA muscle were significantly lower ($p < 0.03$) on R+0 compared with pre-HDBR values (BDC-1) but did not show significant differences in the female or control groups. The last StS test on R+6 indicated that these TA-FTA changes from BDC-1 were sex-dependent ($p > 0.4$) (Table 4-3).

In terms of muscle-pump baroreflex (SBP↔EMGimp) low-frequency (LF) gains, there were significant sex- and intervention-related changes. The LF gains were considerably decreased for all muscle groups in male participants ($p < 0.001$) on R+0, while no significant changes were observed in female participants ($p > 0.7$). This impairment was not recovered in any of the muscles, and the significant reduction persisted for all muscles on R+6 ($p < 0.001$). There were intervention differences among all groups for each muscle in terms of gain (Table 4-3). The gain was substantially lower in control groups on R+0 ($p < 0.001$), while no change from baseline was observed in exercise participants following HDBR in any of the studied groups ($p > 0.10$). This impairment in gain for the control group was not fully recovered on R+6 compared with BDC-1 for all muscles ($p = 0.02$) (Table 4-3).

Table 4-3. Wavelet transform analysis of systolic blood pressure and various studied calf muscle electromyography impulse interactions during standing for different groups including males, females, control and exercise groups on BDC -1 and R+0, and R+6. BDC -1: baseline data collection 1 day before bed rest; R+0: 2 hours after the end of bed rest; R+6: six days after bed rest; Gain: wavelet transform gain; FTA: fraction time active (above significant coherence threshold); LF: low frequency. Values are means (± standard error).

SBP-EMG	Variable	Sex	BDC -1	R+0	R+6	intervention	BDC -1	R+0	R+6
LG	**LF FTA**	**Male**	46.68 ± 4.72	20.03 ± 3.46*	22.34 ±3.31*	**Exercise**	39.12±7.92	17.22±4.86*	25.79±8.85
		Female	47.34 ± 4.94	26.49 ± 5.30*ι	33.60 ±4.68	**Control**	54.89±5.42	29.30±8.92*	30.15±3.96
	LF Gain µV. s/mmHg	**Male**	3.11 ± 2.92	0.13 ± 0.03*#ε	0.16 ± 0.04*#	**Exercise**	0.12±0.03	0.11±0.04‡	0.14±0.06‡
		Female	0.14 ± 0.05	0.08 ± 0.02#	0.08 ± 0.01#	**Control**	3.12±4.29	0.09±0.02*‡	0.09±0.02*‡
MG	**LF FTA**	**Male**	15.73 ± 2.02	14.41 ± 2.49	15.99 ±2.46α	**Exercise**	15.90±3.87	8.53±2.41	12.75±2.96
		Female	19.41 ± 2.65	16.51 ± 3.19ιλ	15.39 ±2.52	**Control**	19.24±3.79	22.38±6.66	18.63±3.85
	LF Gain µV. s/mmHg	**Male**	1.65 ± 1.17	0.30 ± 0.05*#ζε	0.37 ± 0.06*#	**Exercise**	0.31±0.08	0.27±0.07‡	0.41±0.13
		Female	0.28 ± 0.07	0.38 ± 0.11#	0.38 ± 0.08#	**Control**	1.62±1.87	0.40±0.16*‡	0.34±0.09*
TA	**LF FTA**	**Male**	48.15 ± 4.85	20.46 ± 4.48*#	31.88 ±5.74#α	**Exercise**	25.54±6.56	12.24±1.99*	21.12±7.33
		Female	27.18 ± 4.74	20.46 ± 4.32#	29.54 ±5.72#	**Control**	49.80±7.55	28.68±9.34	40.29±8.66
	LF Gain µV. s/mmHg	**Male**	1.11 ± 0.75	0.24 ± 0.04*#	0.27 ± 0.14*#	**Exercise**	0.40±0.17	0.56±0.16‡	0.36±0.21‡
		Female	0.35 ± 0.06	0.50 ± 0.11#	0.22 ± 0.06#	**Control**	0.86±0.82	0.16±0.05*‡	0.13±0.06*‡
S	**LF FTA**	**Male**	35.10 ± 4.79	22.47 ± 4.98	26.33 ±1.06	**Exercise**	32.06±8.77	17.37±5.49	25.58±5.81
		Female	40.23 ± 4.02	24.56 ± 4.44λ	24.45 ±4.86	**Control**	42.27±4.98	29.66±8.23	33.20±8.36
	LF Gain µV. s/mmHg	**Male**	1.80 ± 1.61	0.15 ± 0.02*#ζ	0.21 ± 0.04*	**Exercise**	0.17±0.05	0.16±0.04‡	0.20±0.08‡
		Female	0.15 ± 0.04	0.12 ± 0.02#	0.16 ± 0.05	**Control**	1.78±1.74	0.11±0.03*‡	0.18±0.07*‡

Legend: *: significantly different from BDC-1, †: R+6 different from R+0, ‡: on each day, the control and exercise intervention groups were significantly different for the same sex. Greek symbols: Values with similar Greek symbols were significantly different between the corresponding leg muscles in the same variable in the same group on each day. Significance was set at $p < 0.05$.

Following HDBR, muscle-pump baroreflex (SBP→EMGimp) causality was significantly reduced across all muscle groups in males ($p < 0.001$), but not in females. On R+6, decreased causality in males persisted only in the LG ($p = 0.026$), while data from the other muscle groups showed no significant differences compared with baseline. This reduction in muscle-pump baroreflex causality was primarily observed in control participants. All muscles, except the TA, had a substantial reduction in muscle-pump baroreflex causality in control groups on R+0 compared with pre-HDBR values ($p < 0.001$), while no intervention-related changes from baseline were found in TA ($p > 0.6$). There was no change in muscle-pump (EMGimp→SBP) causality in all studied muscles after HDBR (Table 4-4).

Table 4-4. convergent cross-mapping of systolic blood pressure and various studied calf muscle electromyography impulse interactions during standing for different groups including males, females, control and exercise groups on BDC -1 and R+0, and R+6. BDC -1: baseline data collection 1 day before bed rest; R+0: 2 hours after the end of bed rest; R+6: six days after bed rest; Causality: control directionality; LF: low frequency. Values are means (± standard error).

Causality	Variable	Sex	BDC -1	R+0	R+6	intervention	BDC -1	R+0	R+6
LG	**Causality SBP→EMG**	**Male**	0.92 ± 0.01	0.76 ± 0.02*#	0.80± 0.03*	**Exercise**	0.91±0.01	0.85±0.05‡	0.82±0.04*
		Female	0.89 ± 0.02	0.85 ± 0.05#	0.86 ± 0.02	**Control**	0.91±0.02	0.76±0.05*‡	0.83±0.03
	Causality EMG→SBP	**Male**	0.91 ± 0.02	0.91 ± 0.01	0.89 ± 0.02	**Exercise**	0.89±0.01	0.93±0.04	0.86±0.02
		Female	0.91 ± 0.01	0.90 ± 0.02	0.86 ± 0.02	**Control**	0.91±0.03	0.86±0.04	0.88±0.03
MG	**Causality SBP→EMG**	**Male**	0.80 ± 0.02	0.71 ± 0.04*#	0.76 ± 0.02	**Exercise**	0.79±0.03	0.74±0.06‡	0.75±0.03
		Female	0.81 ± 0.02	0.76 ± 0.04#	0.76 ± 0.03	**Control**	0.86±0.03	0.72±0.05*‡	0.79±0.02
	Causality EMG→SBP	**Male**	0.91 ± 0.01	0.90 ± 0.01	0.89 ± 0.02	**Exercise**	0.90±0.01	0.92±0.02	0.87±0.03
		Female	0.91 ± 0.01	0.90 ± 0.02	0.86 ± 0.02	**Control**	0.91±0.01	0.76±0.05	0.84±0.02
TA	**Causality SBP→EMG**	**Male**	0.91 ± 0.01	0.81 ± 0.03*#	0.84 ± 0.03	**Exercise**	0.89±0.01	0.80±0.04	0.80±0.04*
		Female	0.84 ± 0.02	0.81 ± 0.04#	0.84 ± 0.02	**Control**	0.91±0.01	0.85±0.03	0.88±0.04
	Causality EMG→SBP	**Male**	0.91 ± 0.01	0.92 ± 0.01	0.91 ± 0.01	**Exercise**	0.85±0.03	0.83±0.06	0.81±0.03
		Female	0.92 ± 0.01	0.89 ± 0.03	0.85 ± 0.02	**Control**	0.93±0.01	0.87±0.04	0.89±0.02
S	**Causality SBP→EMG**	**Male**	0.87 ± 0.02	0.75 ± 0.02*#	0.79 ± 0.01	**Exercise**	0.88±0.01	0.79±0.04	0.80±0.01
		Female	0.84 ± 0.02	0.77 ± 0.06#	0.84 ± 0.02	**Control**	0.85±0.03	0.77±0.05*	0.84±0.02
	Causality EMG→SBP	**Male**	0.90 ± 0.01	0.91 ± 0.01	0.89 ± 0.02	**Exercise**	0.86±0.01	0.91±0.01	0.87±0.02
		Female	0.92 ± 0.01	0.88 ± 0.02	0.87 ± 0.02	**Control**	0.89±0.02	0.88±0.02	0.88±0.02

Legend: *: significantly different from BDC-1, †: R+6 different from R+0, Greek symbols: Values with similar Greek symbols significantly different between the corresponding leg muscles in the same variable in the same group on each day. Significance was set at $p < 0.05$.

4.4.4 Postural Sway

The FTA for EMGimp-related interactions with COP (EMGimp↔COPr, lower leg muscle activity with sway) was significantly increased in females for the TA and SOL muscles on R+0 ($p < 0.001$), followed by a substantial decrease back to baseline on R+6 ($p > 0.17$). There was no change in muscle-sway interaction across the different test days for MG and LG, while the SOL differed between the sexes on R+0 ($p = 0.02$) (Table 4-5). These significant LF FTA increases on R+0 in TA and SOL were observed in the control group ($p < 0.01$), while there were no significant changes in the exercise group on R+0.

Both males and females experienced a dramatic reduction in muscle-sway gain in the LG and SOL muscles on R+0 ($p < 0.001$), with this reduction being maintained through R+6 ($p = 0.018$) (Table 4-5). Also, the EMGimp↔COPr gain was significantly reduced in the MG muscle for males, while no reduction was observed in the TA muscle across different sexes ($p > 0.8$). Although both sexes had substantial decreases in gain in LG, MG, and SOL muscles, the changes from BDC-1 differed significantly between sexes, with males showing a significantly higher reduction compared to females ($p < 0.001$). Furthermore, while significant reductions from BDC-1 were observed in both control and exercise groups for all studied muscles on R+0 ($p < 0.01$), the exercise group experienced a significantly higher reduction in EMGimp↔COPr gain on R+0 compared to control participants ($p < 0.03$) (Table 4-5).

Table 4-5. convergent cross-mapping of center of pressure and various studied calf muscle electromyography impulse interactions during standing for different groups including males, females, control and exercise groups on BDC -1 and R+0, and R+6. BDC -1: baseline data collection 1 day before bed rest; R+0: 2 hours after the end of bed rest; R+6: six days after bed rest; Causality: control directionality; LF: low frequency. Values are means (± standard error).

CoP-EMG	Variable	Sex	BDC -1	R+0	R+6	intervention	BDC -1	R+0	R+6
LG	LF FTA	Male	11.70± 0.95	19.73 ± 1.73	12.29± 2.77	Exercise	11.26±1.00	15.88±2.57	11.68±2.33
		Female	10.72± 1.07	14.15 ± 3.63β	10.54± 1.78	Control	12.16±1.52	18.01±4.13	11.15±2.16
	LF Gain μV. s/mmHg	Male	5.22 ± 1.68	0.43 ± 0.22*#	0.28 ± 0.10*#	Exercise	4.82±1.61	0.41±0.26*‡	0.28±0.10‡
		Female	0.59 ± 0.38	0.16 ± 0.08*#	0.21 ± 0.07*#	Control	0.99±0.61	0.18±0.09*‡	0.21±0.07*‡
MG	LF FTA	Male	17.65 ±2.35	23.38 ± 2.38	18.59± 1.90	Exercise	17.92±2.14	20.59±2.00	19.12±2.06
		Female	18.16± 3.52	16.93 ± 1.12α	19.50± 2.94	Control	17.89±3.91	19.72±1.37	18.97±3.06
	LF Gain μV. s/mmHg	Male	15.17± 1.35	0.59 ± 0.16*#	0.62 ± 0.08*#	Exercise	14.88±1.34	0.73±0.28*‡	0.62±0.10*‡
		Female	0.68 ± 0.13	0.54 ± 0.27#	0.52 ± 0.10#	Control	0.97±0.18	0.40±0.05*‡	0.52±0.08*‡
TA	LF FTA	Male	15.95± 1.33	23.82 ± 3.58	12.89± 3.04	Exercise	20.62±2.26	26.46±3.79	17.09±2.63
		Female	19.89± 2.71	33.38±3.77*αβ	16.91± 4.11	Control	13.23±1.52	30.74±3.57*	12.71±4.76
	LF Gain μV. s/mmHg	Male	0.52 ± 3.79	0.38 ± 0.08	0.40 ± 0.15	Exercise	4.38±3.83	0.59±0.12*‡	0.48±0.15*‡
		Female	0.47 ± 0.06	0.41 ± 0.08	0.23 ± 0.03*†	Control	0.41±0.09	0.20±0.04*‡	0.15±0.02*‡
S	LF FTA	Male	12.04± 1.78	16.47 ± 2.95#	11.23± 1.25	Exercise	10.93±1.03	17.04±2.17	13.34±1.54
		Female	12.18± 1.00	27.71± 3.10*#	17.29± 2.40	Control	13.29±1.71	27.14±4.00*	15.18±1.88
	LF Gain μV. s/mmHg	Male	6.17 ± 0.62	0.42 ± 0.18*#	0.34 ± 0.06*#	Exercise	5.94±0.63	0.45±0.16*‡	0.34±0.08*‡
		Female	0.53 ± 0.27	0.20 ± 0.08*#	0.28 ± 0.08*#	Control	0.76±0.27	0.17±0.05*‡	0.28±0.06*‡

Legend: *: significantly different from BDC-1, †: R+6 different from R+0, #: 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, Greek symbols: Values with similar Greek symbols significantly different between the corresponding leg muscles in the same variable in the same group on each day. Significance was set at $p < 0.05$.

No difference in EMGimp→COPr (muscle activity driving sway) causality was observed between the four leg muscles in all studied sex and intervention groups on R+0. However, our results showed that males had a substantial reduction in sway velocity-mediated muscle activity (COPrv→EMGimp) causality in LG, MG, and SOL on R+0 ($p < 0.01$), while females only experienced a significant reduction in MG after HDBR ($p = 0.02$). Furthermore, the results indicated a substantial reduction in this causality for exercise groups within the LG and SOL on R+0 compared to baseline responses ($p <= 0.002$) (Table 4-6). On the other hand, the control group showed a significant reduction in COPrv→EMGimp causality after HDBR on R+0 in both MG and TA ($p < 0.001$). All these notable changes in postural sway-mediated muscle-pump causality persisted on R+6.

Table 4-6. convergent cross-mapping of center of pressure and various studied calf muscle electromyography impulse interactions during standing for different groups including males, females, control and exercise groups on BDC -1 and R+0, and R+6. BDC -1: baseline data collection 1 day before bed rest; R+0: 2 hours after the end of bed rest; R+6: six days after bed rest; Causality: control directionality; LF: low frequency. Values are means (± standard error).

Causality	Variable	Sex	BDC -1	R+0	R+6	intervention	BDC -1	R+0	R+6
LG	Causality EMG→CoP	Male	0.79 ±0.02	0.75 ± 0.04	0.74 ± 0.03	Exercise	0.77±0.02	0.77±0.05	0.70±0.04
		Female	0.76 ± 0.01	0.78 ± 0.03	0.72 ± 0.02	Control	0.78±0.02	0.76±0.05	0.76±0.03
	Causality CoP→EMG	Male	0.90 ± 0.01	0.79±0.03*	0.79 ± 0.03*	Exercise	0.88±0.02	0.78±0.05*	0.80±0.02*
		Female	0.86 ± 0.01	0.78 ± 0.04	0.83 ± 0.02	Control	0.88±0.01	0.78±0.05*	0.81±0.03
MG	Causality EMG→CoP	Male	0.78 ± 0.02	0.74 ± 0.04	0.73 ± 0.02	Exercise	0.78±0.03	0.74±0.05	0.72±0.02
		Female	0.77 ± 0.01	0.75 ± 0.03	0.74 ± 0.01	Control	0.78±0.02	0.75±0.04	0.75±0.02
	Causality CoP→EMG	Male	0.82 ± 0.02	0.71± 0.03*	0.76 ± 0.02	Exercise	0.79±0.03	0.68±0.06*	0.75±0.03
		Female	0.80 ± 0.02	0.70± 0.05*	0.77 ± 0.02	Control	0.83±0.03	0.72±0.05*	0.78±0.02*
TA	Causality EMG→CoP	Male	0.81 ± 0.03	0.79 ± 0.04	0.76 ± 0.03	Exercise	0.83±0.03	0.83±0.05	0.79±0.06
		Female	0.83 ± 0.02	0.82 ± 0.04	0.74 ± 0.06	Control	0.81±0.03	0.78±0.06	0.71±0.07
	Causality CoP→EMG	Male	0.89 ± 0.01	0.81 ± 0.04	0.82 ± 0.03	Exercise	0.86±0.02	0.84±0.04	0.85±0.04
		Female	0.87 ± 0.01	0.84 ± 0.04	0.83 ± 0.02	Control	0.90±0.01	0.81±0.30*	0.80±0.03*
S	Causality EMG→CoP	Male	0.79 ± 0.02	0.75 ± 0.03	0.71 ± 0.02	Exercise	0.78±0.03	0.75±0.05	0.69±0.02
		Female	0.77 ± 0.01	0.77 ± 0.03	0.72 ± 0.02	Control	0.79±0.02	0.76±0.03	0.74±0.03
	Causality CoP→EMG	Male	0.87 ± 0.01	0.75± 0.03*	0.78 ± 0.02*	Exercise	0.83±0.02	0.71±0.06*	0.78±0.02*
		Female	0.81 ± 0.01	0.76 ± 0.04	0.82 ± 0.02	Control	0.85±0.01	0.80±0.05	0.81±0.03

Legend: *: significantly different from BDC-1, †: R+6 different from R+0, Greek symbols: Values with similar Greek symbols significantly different between the corresponding leg muscles in the same variable in the same group on each day. Significance was set at $p < 0.05$.

4.4.5 Baroreflex Active Gain

To provide a clearer understanding of the impact of HDBR on the effectiveness of cardiac and muscle-pump baroreflexes, we calculated the Active Gain (Gain × FTA) on BDC-1 and R+0 for all muscle groups (Table 4-7). In the muscle pump baroreflex system, males exhibited significant reductions in active gain across all studied muscles ($p < 0.001$). Female participants, however, did not show significant reductions in active gain in the MG and TA muscles post-bed rest compared to pre-bed rest responses, but displayed considerable decreases in the LG and SOL muscles ($p < 0.001$). Both exercise and control groups showed significant reductions in active gain on R+0 from baseline values ($p < 0.001$). Regarding postural sway, the MG and TA muscles mirrored their muscle pump baroreflex results, showing no significant changes on R+0 from baseline values in female participants. Additionally, in the MG muscle, not only females but also males and the control groups did not change on R+0, whereas exercise participants exhibited significant reductions ($p < 0.001$). The LG and S also demonstrated significant reductions in the interaction between changes in postural sway and subsequent changes in muscle activity ($p < 0.001$).

Table 4-7. Active Gain (Gain × FTA) of cardiac and muscle-pump baroreflexes before and after bed rest for different groups including males, females, control and exercise groups on BDC -1 and R+0. BDC -1: baseline data collection 1 day before bed rest; R+0: 2 hours after the end of bed rest. Values are means (± standard error).

	SBP-EMG			**CoP-EMG**		
Active Gain	**Group**	**BDC -1**	**R+0**	**Group**	**BDC -1**	**R+0**
LG	**Male**	145.15 ± 13.70	2.60±0.71*	**Male**	61.03±20.19	8.48±4.56*
	Female	6.63±2.32	2.12±0.68*	**Female**	6.32±4.19	2.26±1.22*
	Exercise	4.69±1.23	1.89±0.78*	**Exercise**	54.29±18.23	6.51±4.40*
	Control	171.26±23.71	2.64±0.88*	**Control**	12.04±7.90	3.24±1.82*
MG	**Male**	25.95±18.86	4.32±0.94*	**Male**	267.81±34.95	13.79±3.97*
	Female	5.43±1.50	6.27±2.15	**Female**	12.35±2.54	9.14±4.71
	Exercise	4.93±1.47	2.30±0.77*	**Exercise**	266.83±28.77	15.03±5.92*
	Control	31.16±39.06	8.95±4.35*	**Control**	17.35±3.89	7.89±1.02*
TA	**Male**	53.46±36.81	4.91±1.14*	**Male**	8.29±60.65	9.05±2.02
	Female	9.51±1.78	10.23±2.65	**Female**	9.35±1.38	13.68±2.88
	Exercise	10.22±4.64	6.85±2.17*	**Exercise**	90.32±80.66	15.61±3.47*
	Control	42.83±41.52	4.59±1.65*	**Control**	5.42±1.33	6.15±1.34
S	**Male**	63.18±58.76	3.37±0.79*	**Male**	74.29±11.51	6.92±3.30*
	Female	6.03±1.72	2.95±0.59*	**Female**	6.45±3.31	5.54±2.33
	Exercise	5.45±1.74	2.78±0.94*	**Exercise**	64.92±7.21	7.67±2.83*
	Control	75.24±74.58	3.26±1.00*	**Control**	10.10±3.88	4.61±1.40*

Legend: *: significantly different from BDC-1. Significance was set at $p < 0.05$.

4.5 Discussion

In this pioneering study at the confluence of spaceflight, aging, and physical inactivity, we have shown distinctive responses in lower leg muscle groups among 55-to 65-year-old men and women exposed to fourteen days of continuous HDBR. The current analysis uncovered distinct patterns in the muscle pump baroreflex among the lower leg muscles, along with differences associated with sex and exercise intervention. Diminished control measures like reduced gain, causality, and fraction of time active for the muscle-pump baroreflex were observed.

Our findings revealed a significant decrease in the activity of the LG, SOL, and TA muscles in males, as measured by EMGimp on R+0, with these reductions primarily occurring in the control group. This showed the effectiveness of the proposed exercise intervention in preserving lower leg muscle function following HDBR for male participants. In contrast, females exhibited smaller decreases in both EMG and EMGimp, likely due to inherent physiological differences in muscle composition and mass.

The central overlap component between all systems in the Venn diagram showed a significant increase in females, suggesting a potential adaptation or change in this group. Our research also indicated significantly higher postural sway (COPr) in these older adults following HDBR, especially in females, likely because of impaired muscles requiring more time to generate forces for postural correction, resulting in higher postural sway. This increased sway was associated with greater leg muscle activity in TA and MG, as evidenced with no change in EMG↔COPr active gain. Additionally, this was followed by preservation in EMGimp↔SBP active gain in both TA and MG for females following HDBR. This highlights the interaction between postural sway-mediated leg muscle activation and the muscle-pump baroreflex in maintaining posture and blood pressure, suggesting an adaptation or change in central overlap between the systems in female participants.

These compensatory mechanisms not only emphasize the resilience of the human body in response to challenges like bed rest, but also presents valuable insight into the potential sex-specific differences in muscle, blood pressure, and sway interactive mechanisms. Understanding these intricate responses is crucial, especially in the context of spaceflight, where astronauts experience prolonged periods of physical inactivity, and in aging populations, where maintaining cardiovascular health is paramount for overall well-being.

4.5.1 Orthostatic Intolerance

The new findings of this study highlight significant sex dependent differences in the muscle-functioning and its impact on OI. Males exhibited a significant decrease in the activity of the LG, SOL, and TA as measured by EMG and EMGimp on R+0 (Table 4-1). These significant reductions in muscle activity were primarily observed in the control group and not in the exercise participants. This indicates that the proposed exercise intervention may have the potential to preserve the lower leg muscles following HDBR. There was a smaller decrease in both EMG and EMGimp in females, suggesting that the observed sex differences in EMG reductions could be attributed to inherent physiological differences in muscle composition and mass between males and females. Males typically have greater muscle mass and different muscle fiber type distributions, which might make their muscles more prone to atrophy during periods of inactivity. The varied responses of lower leg muscles in males and females to mitigate the effects of HDBR emphasizes the significance of customizing exercise interventions according to sex.

There were rationales for selecting the type of exercise countermeasures tested in this study [2]; however, this study presents compelling evidence showing that the countermeasures cannot prevent a reduction in the muscle pump baroreflex upon standing in both male and female older adults [34]. These outcomes align with those of prior studies involving bed rest, where lower-intensity aerobic exercise countermeasures were applied in younger adults [17, 182]. Contrary to our initial hypothesis

[84, 183], our results show that high-intensity interval exercise does not yield the same benefits post-HDBR as those observed in maximal exercise [184] or in high-intensity interval training concerning blood pressure regulation among ambulatory older adults not subjected to HDBR [185]. Comprehending the alterations in postural and muscle-pump baroreflexes of distinct leg muscles due to activity or inactivity in older males and females is vital for developing effective countermeasures against OI.

In studies of younger adults, enhancing orthostatic tolerance following bed rest required a combination of exercise with fluid loading or gravity-like stimuli application, both of which are capable of increasing blood volume [17, 186]. Alternatively, maximal intensity exercise was also effective [184]. Despite these interventions, when our findings revealed that both the control and exercise groups experienced a reduction in blood volume during bed rest [85, 86], the beneficial effects of exercise were not evident in this study. This lack of improvement persisted even in individuals with a relatively preserved blood volume. These findings have direct implications for understanding OI, a syndrome affecting both older [34, 187] and younger [36] individuals following HDBR, simulated microgravity (e.g., HDBR) [82, 187], or spaceflight experiences among astronauts [116, 129, 133]. The older age composition of our study participants provides valuable insights into the interplay between age and inactivity concerning the cardio-postural control system.

4.5.2 Skeletal Muscle Pump

The decline in cardiac baroreflex function is a recognized consequence of aging; however, to date, the impact of aging on the muscle-pump baroreflex remains inadequately explored [38]. Impaired baroreflex control and diminished muscle performance contribute to postural instability and orthostatic hypotension, leading to falls in the elderly [118, 188]. Therefore, a thorough understanding of the age-related alterations in muscle-pump baroreflexes is crucial for developing effective countermeasures against orthostatic hypotension. In this study, we exposed older adults to an orthostatic challenge using the StS test and examined the variation in muscle-pump baroreflex for various leg muscles across the four sex and intervention groups before and after bed rest.

Fraction Time Active, signifies the proportion of time during standing in which there is a significant interaction between SBP and corresponding leg muscles, while gain shows the ability of EMG to cause changes in BP. By calculating the active gain (FTA × gain) of the muscle pump baroreflex system, we can monitor the effectiveness of baroreflex responses following HDBR. Our study found that all examined lower leg muscles exhibited a significant reduction in muscle-pump active gain on the first day after bedrest (R+0) compared to pre-bedrest levels across both exercise and control groups. This finding indicates that, although exercise may maintain muscle activity (as measured by EMG and EMGimp) post-bedrest, it does not fully protect the cardio-postural system following HDBR. One potential explanation for this outcome is the absence of orthostatic challenges during the interventions. Without such stressors, the baroreflexes are not activated, leading to a decline in cardio-postural system functionality after adapting to the new condition. Consequently, preserving muscle mass and strength alone was insufficient to maintain cardio-postural system functionality. To ensure orthostatic tolerance is protected in astronauts, it is essential to incorporate orthostatic stressors alongside high-intensity aerobic and strength exercise training during bed rest.

4.5.3 Postural Control

Falls associated with orthostatic hypotension pose a recognized risk of injury in older adults engaged in everyday activities. Relying solely on the assessment of autonomic blood pressure control for evaluating orthostatic tolerance may prove inadequate because of the influence of other physiological systems in regulating blood pressure during upright challenges. Including postural system measurements, specifically leg muscle activation, alongside autonomic control, can offer additional insights into an individual's capacity to maintain blood pressure during orthostatic challenges. Previous research has correlated increased postural sway with increased leg muscular activity in older adults [189, 190]. In this study, we observed a significant closed-loop interaction between EMGimp and postural sway in all leg muscles, whereas the causal relationship of muscle activation inducing postural sway varied among the corresponding leg muscles.

The altered correlation between BP and muscle involvement (including gain, FTA, and causality) between BDC-1 and R+0 showed a potential post-bed rest reduction in reflex output to the muscle and a change in activation. Postural sway was increased in all participants, as observed with increased COPr and COPrv (Table 4-2, Figure 4.2). Furthermore, the Venn diagram suggested that there was a fourfold increase in the interaction between EMGimp ↔COPr and COPr↔SBP zones (denoted by pink color) in females, suggesting that the increase in the postural sway in females led to an engagement of muscles for BP regulation in cardio-postural system (Figure 4.3). This engagement activated all three systems simultaneously and resulted in a significant increase in central overlap in Venn diagram for females. This indicated a potential adaptation or change in these groups not observed in males. Additionally, the increase in postural sway in the females could be a potential compensatory mechanism for maintaining BP during standing. However, it did not translate to an improvement in the muscle pump baroreflex system, as evidenced by the considerable decline in SBP↔EMGimp also seen in the Venn diagram.

Our results showed that the interaction between postural sway and the muscle activities of the TA and MG in females was the only interaction that did not notably decrease following HDBR in all participants (no significant reduction in active gain of the TA and MG on R+0 compared to baseline). This can be explained by its anatomical responsibilities during standing. The TA is primarily responsible for dorsiflexion of the foot, while The MG is primarily involved in plantarflexion. These muscles help to stabilize the ankle and maintain an upright posture by preventing the body from tipping forward and backward. The preservation of EMGimp↔COPr active gain in these muscles implies that the increased postural sway in females led to heightened TA and MG activation. Coincidental a preservation of muscle-pump (SBP↔EMGimp) active gain for the TA and MG in females could signify sway-related augmentation in venous return, improving blood flow back to the heart, which affected cardiac output and consequently blood pressure. These differences in posture-muscle activity in the TA and MG, but not in other leg muscles, could account for the R+0 preservation in TA and MG EMGimp observed in female participants during standing. This phenomenon highlights the intricate interplay among postural stability, muscular activity, and cardiovascular regulation in older female adults, shedding light on potential strategies for mitigating the risks associated with postural instability, such as falls and orthostatic intolerance.

These data can assist in the development of targeted exercise and training techniques to counteract the decline in muscle-pump baroreflexes that occur with age and/or inactivity. The space countermeasures investigated in this study were designed to maintain the health of already healthy older persons [187] when placed in HDBR. Although this will most likely not be the case for hospitalized older patients, they can provide geriatricians and rehabilitation specialists with valuable insight into the musculoskeletal and cardiovascular changes that occur with aging and inactivity. These studies can aid in the creation and development of efficient exercise and rehabilitation programs to evaluate the functionality of key blood pressure regulation mechanisms to combat the impacts of aging, microgravity, and neurological diseases [1, 109, 116].

4.5.4 Space-Based Countermeasures: From Evidence to Practice

Regulations aboard the ISS mandate exercise for crew members on prolonged missions, which prevents the study of the physiological effects of spaceflight in the absence of exercise. Comparing current missions to earlier ones [145] or to periods before significant hardware changes[145, 146] offers the only means to evaluate the effectiveness of current space exercise countermeasures.

Therefore, limited opportunities for controlled intervention studies in space pose significant challenges in developing new exercise countermeasures [12, 147]. In-space exercise intervention studies are costly and time-consuming, and terrestrial exercises like HDBR, while less intricate than space research, offer better experimental control and quicker hypothesis testing. Advances in ISS exercise hardware, without restrictions on time, frequency, or intensity, have facilitated the adoption of terrestrial exercise training concepts such as continuous and interval-type aerobic exercises and high-intensity, multi-set/rep resistance training [151]. Designing optimal exercise countermeasures for spaceflight is complex and requires consideration of several parameters: (a) optimal exercise maintenance mode, volume, and intensity, (b) time-efficient, (c) adequate muscle and bone loading, (d) constraints on hardware mass, and (e) volume and power consumption.

The utilization of a combination of aerobic, HIIT, and resistance exercises in this study did not effectively prevent orthostatic hypotension after bed rest [191], despite the significant preservation of overall beat-to-beat muscle activity during orthostatic challenges [34]. Although EMGimp was conserved in all muscle groups in the exercise groups, the decrease in reflex activity and the causal connection between blood pressure and muscle contractions in certain groups might have diminished the overall effectiveness of the proposed intervention. This unexpected outcome suggests that the enhancement of muscles alone may not be sufficient to counteract the negative effects of inactivity/microgravity. The inability to protect or enhance the baroreflex may have compromised the body's ability to appropriately respond to postural changes, leading to the ineffectiveness of the exercises in preventing orthostatic hypotension. To address this, future interventions may need to incorporate orthostatic challenges during exercise regimens to ensure a more comprehensive approach to mitigating the impact of bed rest on older adults [192].

Tailoring sex-related exercise countermeasures is essential, because of the differences in their muscle-pump baroreflex responses. For males, it is imperative to design an exercise countermeasure that preserves the MG and SOL muscles, considering their significant role in orthostatic challenges. Conversely, for females, special attention should be given to the TA muscles, which are activated during orthostatic challenges to compensate for the effects of inactivity on other muscles. This tailored approach ensures that exercise countermeasures are not only effective but also optimized to support the diverse needs of astronauts, enhancing their overall performance and well-being in space.

It is essential to explore innovative exercise paradigms that not only align with space-based requirements but also address the specific needs of older adults. Integrating emerging technologies, such as virtual reality-assisted exercises and adaptive resistance training, could offer promising avenues for personalized and effective exercise interventions. A comprehensive understanding of the interplay between exercise modalities and intricate physiological mechanisms, considering age- and sex-specific responses, is crucial for advancing our knowledge in the field of aging. However, translation of space-based exercises to older persons in simulated microgravity environments like HDBR requires careful consideration. Older adults exhibit unique physiological responses, including changes in muscle mass, bone density, and cardiovascular function.

4.5.5 Limitations and Future Work

One limitation of this study is the potential interference in EMG recordings due to various factors related to the recording environment and individual differences. Variables such as temperature, humidity, skin impedance, and skin thickness can vary significantly before and after tests, potentially affecting the accuracy and consistency of our results. For instance, changes in skin impedance and thickness can alter the quality of the electrode-skin contact, leading to variations in the recorded EMG signals. While we implemented several measures to minimize these errors—such as standardizing electrode placement, ensuring consistent skin preparation, and maintaining a controlled environment—some variability is unavoidable. Despite our efforts, these factors may still introduce some degree of error in our EMG recordings and subsequent analyses. The statistical comparison of EMG signals between BDC -1 and BDC -5 across all participants, with no significant differences, confirmed the consistency and reliability of our EMG recordings. This validation ensured that our data accurately reflects muscle activity, and any changes after bed rest were due to physiological changes rather than measurement artifacts.

Healthy older adults (55–66 years of age) were recruited for this study, but many older adults already take multiple medications and suffer from significant sarcopenia before being hospitalized [118, 158]. They may spend extended time bedridden due to acute illnesses, serious injuries, surgical procedures, or chronic illnesses. In addition, age-related physiological changes are linked to hormones, exercise levels, food, and disease, making it difficult to identify the core reasons for muscle loss and cardiovascular alterations [115]. In the future, scientists should investigate how different periods of HDBR affect the cardio-postural connections of older individuals. There are substantial sex-related variations and interindividual variability, so future HDBR research should include larger sample sizes in both biological sexes. Breathing, which has been shown to affect both blood pressure and postural stability, was not factored into this analysis [193-195]; therefore, ventilation monitoring should be included in future studies on cardio-postural deconditioning.

Exercising has been identified as an effective method for mitigating the health risks associated with prolonged HDBR [149, 161]; however, evaluating the effectiveness of different types of exercise as individualized countermeasures for males and females requires more research. More research should be conducted to determine individualized training variables, such as loading frequency, workload, pace, rest duration, and specific exercise “dosage.” The cardio-postural model presented in this study did not consider other factors that may alter postural responses, such as visual (eyes closed during testing) or vestibular inputs. Future research should adopt and evaluate a model that accounts for these factors.

4.6 Conclusion

This study extends prior research by exploring the effect of 14 days of HDBR on the muscle-pump baroreflex of LG, MG, TA, and SOL muscles in older male and female adults. The cardio-postural system impairment of older adults' individual leg muscles after HDBR was associated with sex and intervention. As a compensatory mechanism to enhance venous return to the heart, male participants' SOL and MG muscles became more active in the muscle-pump baroreflex following HDBR. Another factor that aided in blood pressure regulation was an increase in postural sway in females following HDBR, which resulted in the activation of TA to compensate for the impairment of other leg muscles. Our results showed that postural sway activated the TA in female participants, whereas the increase in the interaction of male MG and SOL muscles with sway was mediated by the baroreflex. These findings can help develop more effective countermeasures for monitoring orthostatic tolerance using muscle-pump baroreflexes to reduce falls.

Chapter 5. Exercise Countermeasures for Cardio-Postural Deconditioning During Prolonged Bed Rest: A Focus on Sex-Specific Responses and Spaceflight Applications

5.1 Introduction

Long-term space missions induce adaptations to microgravity, that can pose counterproductive or hazardous challenges to astronauts during re-entry into a gravitational environment, such as landing on the moon, Mars, or returning to Earth [1]. The exposure to microgravity impacts cardiovascular, sensory, and motor neuron functions, resulting in changes in cardiovascular and postural responses, along with associated spinal reflexes, ultimately leading to post-flight dysfunctions [1]. One of the noteworthy health and safety issues faced by astronauts after spaceflight is orthostatic intolerance (OI). Following short-duration spaceflights, approximately 20% of astronauts encountered post-flight OI, while a higher percentage of 83% experienced this condition after extended space missions. [196-198].

In the presence of a gravitational field, such as Earth's, blood accumulates in the lower limb vasculature during standing. A reduction in blood pressure activates reflex responses mediated by a hypothesized cardio-postural control center [1]. These responses include an increase in heart rate, vascular resistance (via arterial baroreflex), and skeletal muscle activity (via muscle-pump baroreflex (MP-BR)). The MP-BR reacts to variations in blood pressure by inducing skeletal muscle contractions to prevent blood pooling during standing and maintain blood pressure. However, disruptions in the cardio-postural system after space travel result in a deterioration of its functionality. The extent of deconditioning and the duration required for recovery from microgravity exposure will affect astronauts' capacity to perform physically demanding tasks during exploration missions, including habitat construction and extravehicular activities (EVA).

The authors are unaware of any studies on extended spaceflight without a prescribed exercise regimen. In future NASA Constellation Program missions, crew members will experience microgravity for 4 to 5 days en route to the Moon and during landing, and approximately 200 days during journeys to and from Mars. If exercise hardware is unavailable because of stowage constraints in short missions or malfunctions during Mars missions, then a substantial risk of crewmember deconditioning would exist [199-201]. Consequently, countermeasure programs must establish in-orbit exercise protocols to either maintain or enhance orthostatic tolerance, ensuring the safe execution of tasks during Moon or Mars landings, and minimizing potential adverse effects. Despite decades of research, the development of an effective, non-invasive, and comprehensive countermeasure for mitigating space-induced cardio-postural deconditioning has achieved only partial success [2].

Ascertaining the maladaptation of the cardio-postural system to microgravity and developing effective countermeasures for OI are among the central goals of space physiology research [34]. However, conducting and interpreting human physiology studies during spaceflight pose challenges due to varying adherence to countermeasures, inconsistent dietary practices [202], participation in other scientific experiments, and the interference of specific mission task requirements [203]. Bed rest serves as an established model for investigating changes in physiological function related to spaceflight within a more controlled environment [34, 169, 192]. Notably, most bed rest studies have focused on a young population with a median age of 24.5 years [23], and those involving older adults have typically not included in-bed exercise countermeasures [25]. Yet, the mean age of astronauts for their first and last flights is 40.9 years (maximum: 58.8 years) and 45.3 years (maximum: 61.3 years, excluding John Glenn at 77.3 years), respectively [24].

Older adults are more vulnerable to environmental changes or illnesses due to their limited physiological reserves [204]. Age-related changes in baroreflex function may also contribute to impaired cardio-postural system, as aging leads to increased stiffness in barosensory vessel walls, reduced effectiveness of cardio-vagal autonomic control, and a decline in somatosensory and motor system performance [82, 205]. Conducting bed rest studies among older adults can yield invaluable insights into developing an optimal physical activity as a countermeasure to mitigate the effects of microgravity deconditioning.

In previous analysis of these participants, we reported that a combination of in-bed continuous aerobic exercises (CA), progressive aerobic exercises (PA), high-intensity interval training (HIIT), and resistance exercises applied to 55-to-65-year-old (older) adults during a 14-day Head-Down Tilt (HDT) bed rest intervention was only partially successful in preserving the MP-BR [34]. Despite a significant preservation of beat-to-beat muscle activity during standing post-bed rest, certain sex and/or exercise intervention groups exhibited reductions in active time and reflex causality. The preservation of beat-to-beat muscle activity may not have been as effective because of diminished reflex activity and causal coupling of blood pressure to muscle contractions and heart rate changes in those specific groups [34]. Yet, the evaluation of the effectiveness of diverse in-bed exercise countermeasures in sustaining the muscle pump baroreflex, along with the quantification and comparison of exercise countermeasures for safeguarding cardio-postural responses, remains unexplored.

By employing a head-down bed rest (HDBR) analogue, we have aimed to address pivotal questions regarding the design and optimization of an efficient exercise countermeasure for mitigating physiological deconditioning during spaceflight, specifically focusing on its impact on the MP-BR response. The objectives outlined in this paper with older adults aged 55-to-65 are: 1) To evaluate the efficacy of in-bed physical interventions in activating lower leg muscles to sustain muscle-pump functionality after bed rest, achieved by quantifying the outcomes of the exercises; 2) to delineate the benefits of incorporating space-based physical countermeasures in the development of tailored remedies; 3) to propose novel ideas for countermeasure assessments that could prove beneficial for astronauts during extended spaceflight and planetary exploration missions.

5.2 Methods

The investigation was conducted within the framework of a clinical trial (NCT04964999) established in collaboration with the Canadian Institutes of Health Research (CIHR), the Canadian Frailty Network (CFN), and the Canadian Space Agency (CSA) [85, 86]. The Center for Innovative Medicine (CIM) at McGill University Health Centre (MUHC) spearheaded this initiative, with the overarching aim of understanding the health impacts of inactivity among older adults and investigating how exercise training can mitigate these detrimental effects.

5.2.1 Study Design

The Research Institute (MUHC) conducted the trial, which encompassed four bed rest campaigns wherein 5–6 participants per campaign were placed in continuous 6° HDBR as a ground-based analog to spaceflight. Participants lived at the research facility for the 26 days of the study, which comprised 5 days of pre-bed rest baseline, 14 days of HDT, and 7 days of post-bed rest recovery (Figure 5.1). During the baseline and recovery stages, participants remained on site with the freedom to mobilize and engage in all normal activities of daily living, including prescribed post-bed rest rehabilitation. Throughout the HDT phase, participants adhered to a strict and supervised continuous 6° head-down position (with the allowance of pillows). The study adhered to the principles of the declaration of Helsinki and received ethical approval from the McGill University Research Ethics Board (IRB00010120) and the Simon Fraser University Research Ethics Board. All participants provided informed consent before participating in the study with the right to withdraw at any time.

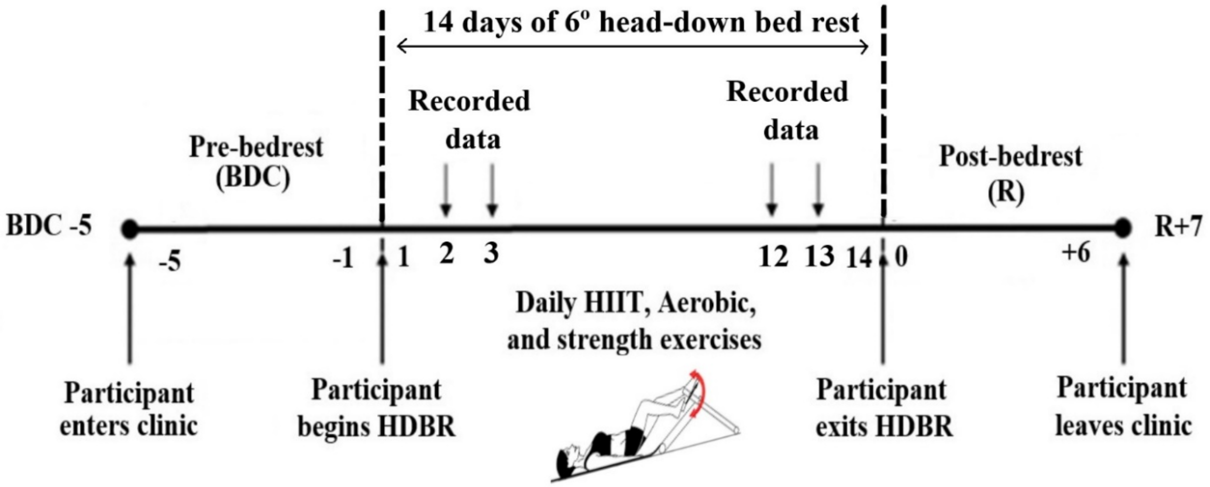


Figure 5.1. Timeline—the participants remained at the testing facility for 26 days, of which 14 days were spent in 6° head-down tilt (HDT). Participants arrived at MUHC 5 days prior to entering HDT. At this time, baseline data collection (BDC) was performed. Then they stayed in bed for 14 days and the intervention sessions were recorded on HDT2, HDT3, and HDT11 and HDT12. After bed rest, the participants remained at the clinic for 7 days where recovery (R) data were collected.

5.2.2 Participant Information

Out of 219 healthy volunteers, and of the 80 initially considered eligible, 56 subsequently declined participation. Ultimately, 24 participants were enrolled in the study, with one exclusion because the designated group reached full capacity. Of the enrolled participants, 20 completed the entire study. One participant withdrew on day 3 of head-down bed rest (HDT3), primarily citing convenience issues (challenges in maintaining the required position, difficulties in bowel movement, and the need for assistance with basic needs). Two participants withdrew from the study on day 3 of the recovery phase (R+3) with an occurrence of atrial fibrillation.

Twenty healthy older adults (10 female; age: 59 ± 3 yr; weight: 70.1 ± 14.2 kg; height: 167 ± 9 cm; and body mass index: 24.9 ± 3.0 kg/m2) voluntarily participated in this 14-day 6° HDBR study. Participants underwent screening for inclusion criteria provided by the CIHR, which were based on age-appropriate modifications to the Guidelines for Standardization of Bed rest Studies [206]. To be eligible, participants had to be habitually active, engaging in at least 2.5 hours of moderate-to-vigorous aerobic activity per week in the month preceding enrollment. They were also required to be non-smokers, not taking any prescription medications, and in good health with no history of chronic illness (all determined by self-report). To minimize the potential risk of thrombosis, staff screened against personal or family history of deep vein thrombosis. To diminish the likelihood of hormonal effects influencing the outcomes of the study, female participants were at least 1 year postmenopausal, with a serum follicle-stimulating hormone level exceeding 30 IU/L.

The exclusion criteria comprised: a) History of heart attacks, thrombosis risk, severe allergies, hypocalcaemia, uric acidemia, orthostatic intolerance, vestibular disorders, significant musculoskeletal issues, chronic back pain, head trauma, seizures, ulcers, renal stones, gastro-esophageal reflux disease, or renal function disorder, hiatus hernia, migraines, or diagnosed psychiatric conditions; b) Electrocardiogram abnormalities; c) Diagnosis of AIDS, hepatitis B, or C; d) Anemia, defined as ferritin lower than 10 or >154 ng/ml (females) and lower than 20 or >245 ng/ml (males); e) Family history of thrombosis; f) Bone mineral density lower than 2 standard deviations of T-score; g) Claustrophobia; h) Special dietary requests (e.g., vegetarian, vegan); i) Contraindications to undergoing magnetic resonance imaging; j) Donated blood in the past three months; k) Smoked (tobacco and/or marijuana) within six months prior to the start of the study; l) Abused drugs, medicine, or alcohol (i.e., >10 drinks a week, with >2 drinks a day most days) within up to 30 days prior to the start of the study; m) Participated in another study within 2 months before the study onset; or n) Positive COVID-19 test within one week to 24 hours before the study start date.

5.2.3 Data Collection

Electrocardiograms (ECG) were recorded using a bipolar three-lead ECG (IX-BIO4, iWorx, USA) with electrodes in a standard Lead III setup. Transdermal recordings from four lower leg muscles—the tibialis anterior, lateral soleus, and both medial and lateral gastrocnemius—were taken with the Bagnoli-8 surface electromyography (EMG) system by Delsys Inc, MA, USA. The EMG sensors are internally shielded to prevent external electrical interference and feature contacts made of 99.9% pure silver bars (10mm length, 1mm diameter, 10mm apart) for optimal signal capture and consistency. The sensor casing's curved design improves skin contact and adhesion, reducing the negative impact of sweat during exercise. For accurate signal detection, sensors were positioned parallel to the orientation of the muscle fibers underneath and placed centrally on the muscle belly, away from tendons and edges. The Delsys Adhesive Sensor Interface ensures a secure attachment, minimizing motion artifacts and line interference. Proper attachment involves removing excess hair, cleaning the skin and sensor with alcohol to remove oils and residues, and allowing them to dry completely before applying the interface.

To ensure reliable EMG signals across different test days despite variations like subcutaneous tissue thickness and electrode-skin impedance, several methodological controls were applied. Electrode placement was standardized using anatomical landmarks and recommended positions by the SENIAM project (Surface Electromyography for the Non-Invasive Assessment of Muscles). Repeatability and reliability were assessed by comparing results from five familiarization test days and one initial StS test day before bed rest. A standard Student's t-test with a Bonferroni correction of the mean value of filtered, rectified, normalized and enveloped EMG signals showed no significant differences within participants and between groups on these days. These controls ensured that differences in EMG signals pre- and post-bed rest were due to physiological changes rather than measurement artifacts. Data were recorded using the National Instruments USB-6218 16-bit data capture hardware and LabVIEW 2013 software at a sampling rate of 1 kHz (National Instruments Inc, TX, USA).

5.2.4 Supine-to-Stand Test Procedure

The supine-to-stand (StS) test was performed twice during BDC and twice during the recovery period to evaluate and stimulate the cardio-postural control system [37, 53, 88-91]. This assessment was scheduled for the mornings of BDC-5, BDC-1, R+0, and R+6, as illustrated in Figure 5.1. Because the StS test was not part of the screening phase, the first session on BDC-5 served as an introductory session for participants. It is important to mention that the StS tests on BDC-1 and R+0 were performed an hour after completing the Canadian Space Agency (CSA) standard tilt test, which lasted no longer than 15 minutes.

For the StS test, a windowless, quiet room was chosen to prevent auditory and visual stimuli from influencing the participants. Upon their arrival, participants were laid down in a 6º Head Down Tilt (HDT) position and equipped for physiological monitoring. After setting up the equipment, the room lights were turned off, and participants were instructed to close their eyes for a continuous data collection period of 5 minutes. Then, they were instructed to open their eyes before being helped into a standing position by the research team—one researcher would move their legs off the bed while another assisted in lifting their upper body. While standing, participants were instructed to place their feet parallel, 5 cm apart, keep their eyes closed, arms relaxed by their sides, maintain a level gaze as if at eye level, and not to move their feet for the next 6 minutes [92]. Participants who were able to complete the standing portion of the StS test on R+0 were labeled as “finishers,” whereas those unable to complete the test due to issues like blood pressure drops, dizziness, or fainting were termed “non-finishers”.

5.2.5 Experimental Groups

This study was an open-label, non-blind, parallel-group proof-of-concept randomized controlled trial. Participants were randomly assigned to one of two experimental groups: 1) HDBR without exercise training (CON); or 2) HDBR with daily bouts of exercise training (EX). Throughout the 14-day HDT protocol (from days 6 to 19), participants in both the EX and CON groups maintained a 24-hour per day 6° head-down tilt bed position with all activities, including eating and personal hygiene, conducted in the head-down bed rest position. Despite sharing the same facility, to prevent contamination, participants from different groups did not interact with each other.

Exercise Group

The EX-participants (6 female and 5 male) underwent three daily sessions of HIIT, progressive aerobics (PA), CA, or lower and upper body strength training exercises. The total daily exercise duration ranged from 60 to 72 minutes per day to address losses in cardiovascular fitness and muscle strength (refer to Table **5-1**). Participants performed all exercises in a 6° head tilt-down tilt position with data collected on day 2 (HDT2), day 3 (HDT3), day 11 (HDT11), and day 12 (HDT12) of head-down bed rest, encompassing various exercise interventions (Table **5-1**). The exercise sessions were scheduled between 6 AM and 6 PM, with a 4-hour interval between sessions. Each session was conducted by a trained exercise instructor with a one-to-one supervision ratio, and no specific motivation strategies were employed. While not discussed within the scope of this paper, nutritional intake was controlled according to the Guidelines for Standardization of Bed rest Studies in the Spaceflight Context to help maintain body weight [206].

The predetermined countermeasure exercise intervention program designed by the CSA [187] aimed to address several physiological systems affected by bed rest or spaceflight. In summary, including HIIT in the protocol had multiple justifications. HIIT has known positive effects on maintaining or improving aerobic fitness. Cassidy et al. [207] demonstrated that HIIT induces robust metabolic and cardiovascular benefits in clinical populations with type 2 diabetes, while Convertino [208] reported potential benefits on baroreflex response. However, the effects of HIIT itself have shown variability, with some studies showing benefit [185] and others showing no effect [209]. The cycling exercises and upper- and lower-body resistance protocols were selected to emulate high-intensity, low-volume programs that have shown partial success in preserving musculoskeletal and cardiovascular outcomes during spaceflight and HDBR, particularly in a younger population [210, 211].

Table 5-1. Bed rest exercise protocols. A combination of up to three per day was performed with a maximum total time of 60-90 minutes per day. The red intervention sessions represent the sessions in which we collected data.

Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Upper Resistance Progressive Aerobic HIIT	Continuous Aerobic(30) Lower Resistance Progressive Aerobic	Progressive Aerobic Continuous Aerobic(15) HIIT	Continuous Aerobic(30) Upper Resistance Progressive Aerobic	HIIT Progressive Aerobic Lower Resistance	Continuous Aerobic(30) Upper Resistance Progressive Aerobic	HIIT Continuous Aerobic(15) Progressive Aerobic
Day 8	**Day 9**	**Day 10**	**Day 11**	**Day 12**	**Day 13**	**Day 14**
Continuous Aerobic(15) Upper Resistance Progressive Aerobic	HIIT Continuous Aerobic(15) Progressive Aerobic	Continuous Aerobic(30) Lower Resistance Progressive Aerobic	HIIT Continuous Aerobic(15) Progressive Aerobic	Continuous Aerobic(30) Lower Resistance Progressive Aerobic	HIIT Progressive Aerobic Upper Resistance	Continuous Aerobic(15) Continuous Aerobic(30) Progressive Aerobic

Exercise type	Exercise windows with effort%			Total exercise time
Continuous Aerobic (15)	3 min warm-up (40% HRR)	9 min steady-state (60%-70% HRR)	3 min cool down (40% HRR)	15
Continuous Aerobic (30)	5 min warm-up (40%-50% HRR)	20 min steady-state (60%-70% HRR)	5 min cool down (40% HRR)	30
HIIT	5 min warm-up (40% HRR)	11 High intensity intervals (30 seconds @ (80-90%HRR)) followed by (1.5 min relax @ (30%-40% HRR))	5 min cool down (40% HRR)	32
Progressive Aerobic	3 min warm-up (30% HRR)	3 min each 40%, 50% and 60% HRR consecutively	3 min cool down (40% HRR)	15
Lower Resistance	Exercises: Hip raise, leg press, ankle pump, leg curls 3 sets (1 warm-up) of 10-12 repetitions			25

The intensity of cycling exercises (CA, PA, and HIIT) was individually determined based on participants' resting heart rate (RHR), heart rate max (MHR), and heart rate reserve (HRR), with HRR calculated as HRR = MHR - RHR. The progression of intensity for both cycling exercises and strength training components was individually tailored, considering the participant's performance and tolerance.

Given that there were two CA training sessions, one lasting 30 minutes and the other 15 minutes, our team exclusively gathered data during the 15-minute CA session to ensure it was comparable to the PA, which also had a 15-minute training period. In PA and CA (15 min) training sessions, participant heart rate and HRR target intensity were continuously monitored. The instructor also tracked resistance, speed, and the Borg Rating of Perceived Exertion (RPE Borg) in minutes 0, 4, 6, 9, 12, and 15 during these sessions. With HIIT sessions, the instructor tracked heart rate and HRR target intensity. The parameters of resistance, speed, and RPE Borg were recorded at minute 0, every minute from 5.5 to 26.5 minutes, and at minutes 27 and 32. The instructor assessed whether the training was executed according to the prescribed protocol at the conclusion of all sessions, and there was not any deviation from the expected adherence and fidelity to the intervention. Also, no adverse events occurred during the exercise sessions. During each strength training session, the exercise instructor recorded the number of repetitions per set, the load used, the participant's rate of perceived exertion (RPE) based on the 10-point Borg RPE scale, and whether the training was conducted as prescribed.

Control Group

Control participants received daily 15–20 minutes physiotherapy sessions, during which a physical therapist administered passive exercises, including stretching, motion therapy, and massages. Participants could engage in activities such as using their laptops, reading, watching television, moving in bed, and performing stretching exercises.

5.2.6 Data Analysis

The analysis focused on the prescribed ergometer exercise sessions. Three out of the four exercises outlined in this study involved the use of a cycle ergometer. The intensity of these exercises was determined based on participant heart rate. In contrast, the lower body strength exercise used cables, resistance band, and body weight for exercising. The intensity was established solely based on participant tolerance. Given the limited standardization in strength training, comparing muscle activity across different participants presented challenges. Therefore, the focus of the analysis was on the ergometer workouts to assess their effectiveness.

Muscle Activity

As surface EMG sensors can lose their connection during testing, they may record noise instead of muscle activity, so manual validation was required before using the specific muscle EMG data. We then applied band-pass filtering to reduce noise and artifacts. A high-pass filter was chosen on the low-frequency end to eliminate slow variations caused by movement artifacts. Typically, a high-pass filter between 10 and 20 Hz retains important surface EMG frequencies, though the 5-20 Hz range also contains information on active motor unit firing rates [87]. However, to minimize estimation uncertainty and account for the low frequency response of cardio-postural control (<0.5 Hz) [127, 212], we used a cutoff frequency of 5 Hz for the EMG envelope extraction filter. On the high-frequency end, approximately 95% of surface EMG power lies within harmonics up to 400 Hz, with additional power from electrode and equipment noise [87]. A low-pass filter was used to further reduce these unwanted components, with a cut-off frequency set at 400 Hz, based on our sampling rate of 1000 samples per second, following the sampling theorem which requires sampling at more than twice the highest signal frequency.

Rectified EMG signals from individual leg muscles (LG, MG, TA, SOL) were added together to obtain aggregate EMG to represent overall muscle activities [37, 89]. Our research focused on investigating the activation of lower leg muscles by the various exercises aimed at preserving the baroreflex system during periods of bed rest. As the muscle activities in both legs work together to respond to orthostatic stresses and regulate blood pressure, we combined the bilateral EMG values to analyze the activation of all muscles during peddling. It is worth mentioning that the surface EMG electrodes employed in this study were not sufficiently sensitive to distinguish between the activities of distinct motor units in the lower leg muscles.

EMG signal analysis methods vary greatly among scientific groups. Standardizing these methods is crucial for accurately comparing EMG activity in the same muscle on different days or across different individuals. To normalize EMG signals, we divided the EMG signals recorded during an exercise session by the maximum activation levels obtained from the same muscle before starting each exercise session. Participants performed ankle plantar flexion and ankle dorsiflexion while their foot was secured in an ergometer bike, with their knee and hip flexed at 90° (in a biking position). Maximal voluntary isometric contractions (MVIC) were performed, with 10-minute intervals between attempts to minimize fatigue, and the highest value was used for normalization.

The area under the EMG envelope curve (AUC) is a widely accepted measure of muscle activity that integrates both the intensity (amplitude) and duration of muscle activation over time [213, 214]. The AUC represents the cumulative electrical activity of the muscle, reflecting motor unit recruitment and firing rates during contraction [213]. This metric is particularly useful for assessing endurance or sustained muscle activities, as it accounts for both the strength of the signal and the duration of activation. By integrating the signal over time, the AUC provides a more comprehensive view of muscle performance than peak amplitude alone, which only captures the maximal intensity of activation [214]. To ensure valid comparisons of muscle activity across exercises of different durations, the EMG AUC was normalized by dividing it by the length of the exercise windows. This normalization adjusts for variations in exercise duration, allowing for comparisons based on the relative intensity of muscle activation rather than total accumulated output. Such an approach is critical for ensuring that differences in total time do not obscure the underlying differences in muscle activation intensity.

The muscle mass in the lower legs varies from person to person, affecting the EMG AUC measurements, which could lead to inaccurate comparisons. To address this, we normalized the EMG AUC values based on the lower leg muscle mass for each participant separately, using data from DXA (Dual-energy X-ray Absorptiometry) scans taken on two test days, HDBR 03 and HDT12. This normalization process provided an average normalized EMG AUC per kilogram of muscle, enabling more accurate comparisons of muscle activity between groups. We normalized all values to a range of zero to one to enhance their graphical representation. The value of one corresponds to the sum EMG of all lower leg muscles recorded during the maximum voluntary contraction test conducted.

Training Impulse

Quantification of factors influencing the efficiency and effectiveness of exercise interventions provides a comprehensive understanding of the effects of different exercises, including CA, PA, and HIIT, on the activation of lower leg muscles. The quantification model takes daily training loads as input. The following general form can express the exercise session training load:

$$\text{training load} = \text{intensity} \times \text{duration}$$

Duration is straightforward; however, quantifying intensity poses a challenge because of the nonlinear relationship between work rate and the resulting metabolic stress, which primarily determines the adaptive stimulus. This nonlinearity is evident in the exponential increase of blood lactate levels as a function of work rate [215, 216]. It is challenging to quantify and compare workouts of varying volumes and intensities in terms of their ability to induce physiological adaptations. Various metrics exist for estimating training load, such as session rating of perceived exertion, ordinal categorization, Lucia’s training impulse, summated heart rate (HR) zone score, and excess postexercise $\dot{V}o_2$ [217-219]. However, the most well-known system for training quantification is Eric Banister's training impulse (TRIMP) [220]. Built on HRR as a measure of intensity, TRIMP considers the observation that higher workloads are metabolically more demanding, and this demand increases exponentially compared with workloads performed for the same duration at lower intensity [221]:

$$\text{TRIMP} = t \times \text{FHRR} \times k$$

$$\text{FHRR} = (\text{HR}\ \ \text{rest}) / (\text{HR max} - \text{HR rest})$$

Where t is the duration of the exercise bout (in minutes), FHRR is the fraction of the heart rate reserve, and k = 0.64 x e^ (1.92 x FHRR) for men or 0.86 x e^(1.67 x FHRR) for women. The ECG was used to calculate heart rate during exercise sessions at different intensities.

Statistical Analysis

In this study, we used a nonparametric ANOVA-type statistic (nparLD, F2-LD-F1 design) suggested by Brunner et al. [222] to examine the impact of finishing the stand section in the StS test on R+0 (finisher, non-finisher), different types of exercise interventions (CA, PA and HIIT), and test days (HDT3, and HDT12), as well as their interactions, on the response variables. The limited sample size of both male and female participants who finished or did not finish the StS test makes it potentially unfeasible to conduct statistical analysis to explore the relationship between sex and whether participants completed the StS test. We used multiple comparisons (LD-F1 design) to study the pairwise differences between BDC-1 and R+0, as well as between HDT3 and HDT12 (time main-effects). To investigate the differences between males and females in the exercise/control and finisher/non-finisher groups at HDT3 and HDT12, the Kruskal-Wallis test was performed, followed by the Conover-Iman post-hoc test. The statistical tests were performed using R [223] with significance set at $p<0.05$ and trends at $0.1>p\geq0.05$.

5.3 Results

This study reports the lower leg muscle activity and exercise findings after 14 days of HDBR in 55-to 65-yr-old men and women as part of the Canadian aging and inactivity study. These include the lower body cycling exercise interventions conducted in-bed: continuous and progressive aerobic, and high-intensity interval training. These protocols were based on a function of participants' heart rates, enabling us to conduct statistical analyses among our measured variables. This research does not include the results of lower body resistance exercises because they were tailored to participant preferences and tolerance and not standardized to physiology.

One notable limitation of this study is the small sample size in the exercise groups for each sex (n = 5), which may not provide sufficient statistical power to accurately represent the broader population. We acknowledge that larger sample sizes are generally preferred for more robust statistical analysis. However, financial, time, and ethical constraints restricted our ability to include more participants, especially considering the potential detrimental effects of prolonged bed rest on physiological systems, particularly in older adults. As a result, the statistical analyses conducted in this study may not fully capture the variability and trends present in the wider population. Nevertheless, these findings could contribute valuable insights into addressing knowledge gaps related to designing exercise countermeasures for space missions. This study marks the first investigation of HIIT in older adults during bed rest, including in-bed exercise countermeasures. Future studies with larger cohorts are needed to validate and expand upon these results.

5.3.1 Aerobic Exercises

To ensure the validity of our EMG measurements for all lower leg muscles and maintain high-quality data recording in line with SENIAM project recommendations, we compared EMG signals from BDC-5 and BDC-1. This comparison assessed the variability in the recorded data. A Student's t-test with Bonferroni adjustment was conducted to compare the average EMG signals over two days for all participants while standing, revealing no significant differences ($p > 0.27$).

There were no significant changes in the EMG AUC between different testing periods (HDT3 and HDT12) during bed rest for males, while female participants showed a significant decreased normalized muscle activity ($p = 0.041$) (Figure **5.2**). These data revealed a higher trend of muscle activity during CA exercises in female participants (60%-70% of HRR) than in male participants ($p < 0.08$) on both test days (Figure **5.2**-a), although there were no significant changes observed in this difference from day 3 to day 12 ($p > 0.7$). Males had a lower TRIMP than females for a comparable EMG AUC (Figure **5.4**). The study also revealed a significant difference in TRIMP between sexes on HDT3 ($p = 0.02$); however, this difference was not evident on HDT12 or on the changes from day 3 to day 12 ($p > 0.8$) across sexes. Kinesiologists continuously adjusted the power output of the ergometer bike to reach the targeted HRR during the different exercise windows [85, 86] (Table **5-2**). To facilitate the comparison of power output across various test days and between sexes, the power output was normalized according to the maximum TRIMP value for each exercise module, considering variations over different days and sexes. This normalization allows for a consistent comparison of the mechanical stimuli exerted on muscles, while ensuring an equivalent cardiovascular load across different test days and sexes (Table **5-2**). The EMG AUC results between male and female, as well as between different test days (HDT3 and HDT12) and between finisher and non-finishers are presented in Figure **5.4**-a.

Table 5-2. Mean (± standard error) power output (watts) of aerobic exercises (continuous and progressive) during different exercise windows for males and females. For continuous aerobic exercises, the power output was recorded during the warm-up, 60-70% heart rate reserve (HRR), and cool-down windows. For progressive aerobic exercises, the power output was recorded during the warm-up, 40% HRR, 50% HRR, 60% HRR, and cooldown windows. HDT3: head-down tilt day 3; HDT12: head-down tilt day 12.

Exercise Mode		HDT3					HDT12				
Continuous Aerobic (watts)	**Male**	76.5±5.2	137.7±18.6			92.7±25	74±4	153±23.3			77.4±4.4
	Female	51.6±5.4	56.6±12			50±4.4	48±6.5	82.8±9.6			48±6.5
Progressive Aerobic (watts)	**Male**	69.6±2	104±9.4	129±20	144±22.1	73.6±4.9	90±4.4	137±18.6	157±22.1	174±26.5	90±4.4
	Female	50±5.1	65±4.3	75±5.6	85±5.6	47.5±6.3	51.6±4	69.1±6.1	79.1±6.1	90.8±6.3	51.6±4

Analysis revealed no differences in the AUC for EMG measurements between males and females during AP exercise on both test days (Figure 5.2-b). Both males and females showed a notable decrease in EMG AUC on HDT12 compared with HDT3 (Figure 5.2-b) ($p < 0.001$), with the changes being more conspicuous in males. Similar to continuous aerobic exercise, there was not a difference in TRIMP values on both HDT3 and HDT12 across sexes ($p > 0.7$). Significant changes in ergometer power output were observed during aerobic exercises (CA and PA) across different sexes on both test days ($p < 0.2$), with males displaying a lower difference between HDT12 and HDT3 than females. Our results showed that the power output required to reach the targeted HRR during CA significantly increased on HDT12 compared with HDT3 for both males and females ($p < 0.03$), with no changes in power outputs for PA across different days in either sex. Among finisher participants, there was a significant increase in TRIMP on HDT12 compared with HDT3 ($p < 0.001$). Non-finishers displayed a decrease in EMG AUC on HDT12 (60% heart HRR) compared with the responses on day three of HDT ($p < 0.001$).

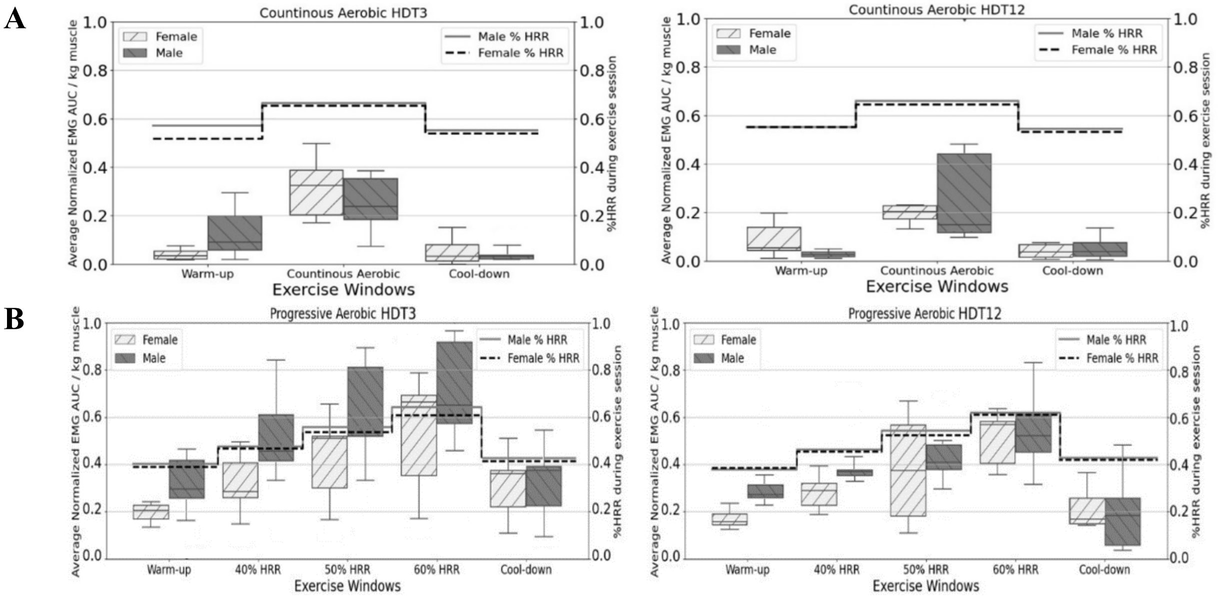


Figure 5.2. Area under the curve for continuous and progressive aerobic exercises, along with heart rate reserve percentage during different testing periods (HDT3 and HDT12) for male and female participants.

5.3.2 High-Intensity Interval Training

The ergometer power output during various exercise windows for HIIT was recorded for both male and female participants (Table 5-3). The power output data were normalized for accurate comparison based on maximum TRIMP values specific to HIIT, compiled over different days and sexes. Unlike aerobic exercises, no significant differences were observed between the sexes on both HDT3 and HDT12 ($p > 0.6$). However, a significant reduction in power output during high-intensity intervals was noted for both males and females on HDT12 compared with HDT3 ($p < 0.04$).

Table 5-3. Mean (± standard error) power output **(watts)** of high intensity interval training during different exercise windows for both males and females. HWR refers to High Work Rate, while RP indicates the Recovery Period between intervals in High-Intensity Interval Training. HDT3: head-down tilt day 3; HDT12: head-down tilt day 12.

		HDT3				**HDT12**			
Exercise Mode	**Exercise Windows**	**Warm-up**	**HWR**	**RP**	**Cool-down**	**Warm-up**	**HWR**	**RP**	**Cool-down**
High Intensity Interval Training (watts)	**Male**	95±4.4	293±17.3	124.6±6	93±4.1	76±4.4	227±11.5	111±1.6	87±4.4
	Female	81±5.4	303±12.8	138±7.6	81±5.5	93±4.9	246±19.8	121±3.5	79.6±5.5

Lower leg muscle activation across different test days was not significantly affected by HIIT for both sexes, with no difference in EMG AUC between the third and twelfth days of bed rest ($p > 0.4$) (Figure 5.3). Although there were no significant changes in EMG AUC on HDT3 across sex, our results showed a significant reduction in EMG AUC in males compared with females on HDT12 during high-intensity intervals ($p = 0.02$). As the HIIT sessions progressed, participants, regardless of sex, showed an increase in muscle activation during recovery periods between HIIT intervals. This increase showed a trend toward significance in females ($p = 0.07$) (Figure 5.3). There was no significant difference in overall muscle electrical activity between the early and late stages of bed rest (HDT3 and HDT12) during high-intensity intervals, and between finisher and non-finisher participants. The other measured variables showed no significant differences.

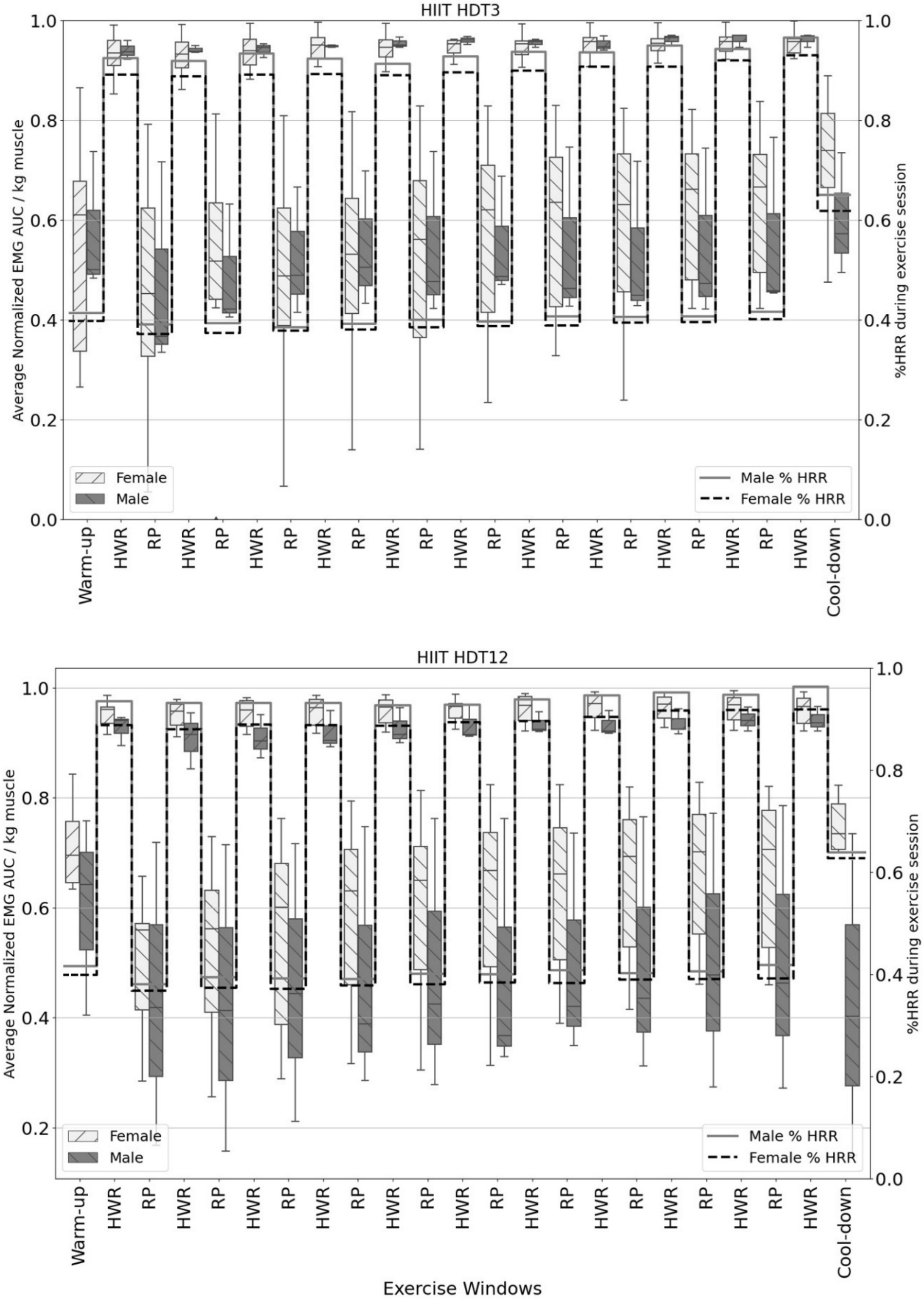


Figure 5.3. Area under the curve for high-intensity interval training, along with the heart rate reserve percentage during different testing periods (HDT3 and HDT12) for male and female participants. HWR stands for high work rate, and RP signifies the recovery period between intervals in high-intensity interval training.

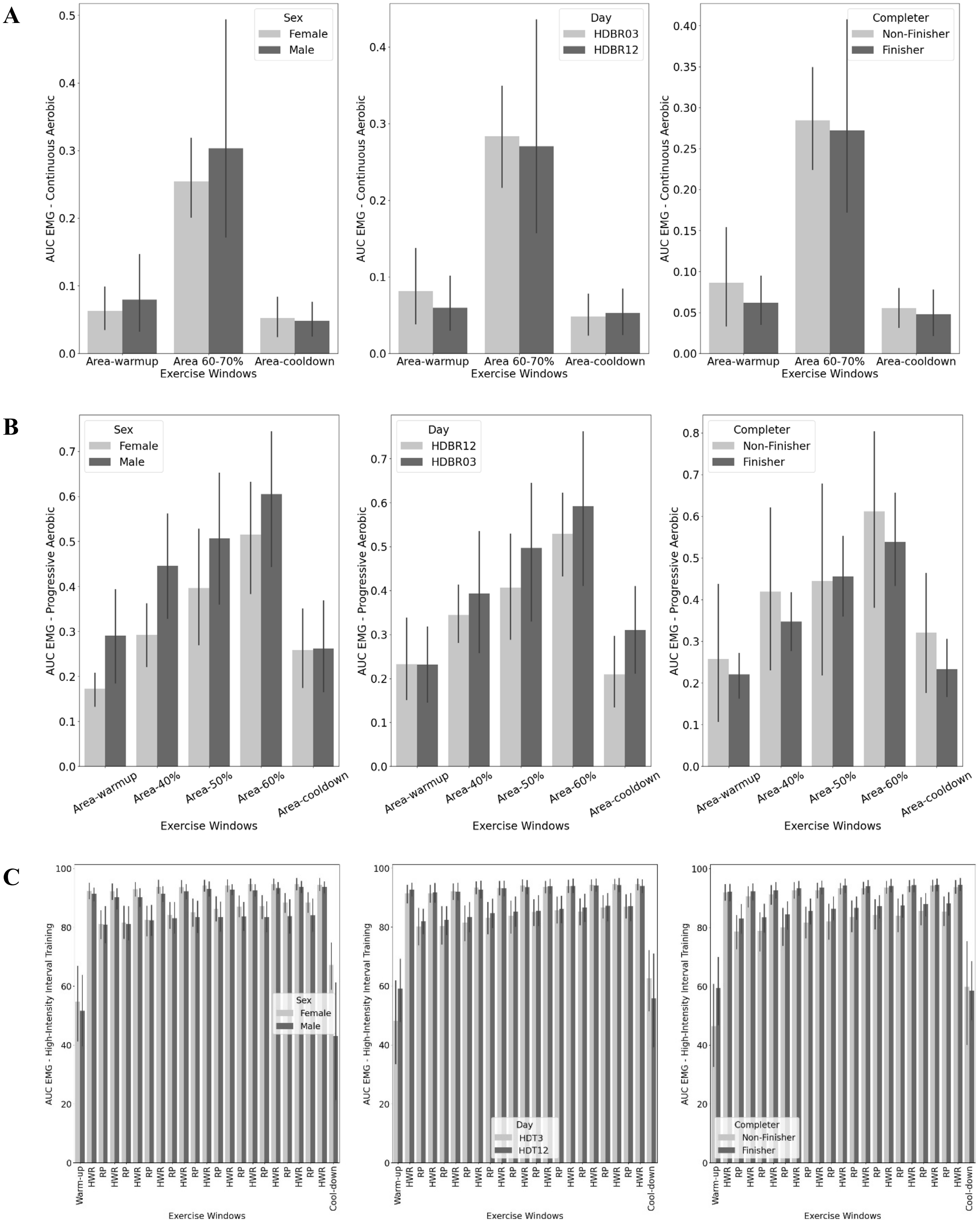


Figure 5.4. Comparison between the area under the curve for various exercise intensities in both continuous, progressive aerobic exercises, and high-intensity interval training, across different sexes, testing days and finisher/non-finisher participants.

5.4 Discussion

This study highlights significant sex-related differences in power output and muscle activation during various exercise interventions. While females exhibited lower power output during continuous and progressive aerobic exercises compared with males, no disparity was observed in HIIT. These differences can be attributed to physiological and biomechanical factors, such as muscle mass, cardiovascular capacity, and body composition. Notably, normalizing muscle activity by muscle mass reduced sex differences, suggesting that HRR could effectively simulate muscular responses across sexes. The study observed consistent HRR targets and variations in EMG activity across different exercise modalities, with higher EMG values during dynamic exercises like PA and HIIT due to increased muscle fiber recruitment, especially in females. Prolonged bed rest led to reduced EMG AUC and increased variability, which was influenced by muscle atrophy, neural adaptations, and cardiovascular deconditioning.

Although exercise countermeasures were beneficial for maintaining overall cardiovascular health and muscle tone, the lack of orthostatic stress during head-down bed rest limited the efficacy of baroreflex preservation. This limitation underscores the need for innovative countermeasures that can introduce orthostatic stress or simulate gravitational forces to safeguard the baroreflex system more effectively during prolonged periods of bed rest or microgravity exposure. Future research should explore the integration of prescribed exercise interventions and orthostatic stressor devices (e.g., artificial gravity or lower body negative pressure) to enhance exercise effectiveness and baroreflex function for males and females, which is crucial for space mission success and health outcomes.

5.4.1 Sex Related Responses

The bed rest study with exercise intervention revealed a significant difference in power output with sex during continuous and progressive aerobic exercises, with females exhibiting lower power output than males. However, this disparity was not observed during high-intensity interval training (HIIT), where power output was comparable between the sexes. Physiological and biomechanical differences could explain this phenomenon. Males generally have higher muscle mass, greater cardiovascular capacity, and larger lung volumes than females. These factors contribute to a higher power output in continuous and progressive aerobic exercises, which primarily rely on sustained and moderate-intensity effort. However, HIIT involves short bursts of high-intensity effort followed by recovery periods, tapping into anaerobic energy systems and muscle power. The shorter, intense bursts rely more on anaerobic capacity and the ability to exert maximum effort in a short time, which does not differ as dramatically between sexes as aerobic capacity does, resulting in more comparable power outputs between males and females.

Biomechanical and anatomical differences between sexes, including variations in body composition, muscle mass distribution, and limb length, can affect the way in which males and females perform exercises. Although muscle mass varies between individuals, the commonly higher muscle mass in males, results in a larger recruitment of muscle, and higher EMG readings compared with females. In this study, we noted a significant sex-related difference in muscle activation during exercise sessions. However, normalizing for muscle activities based on muscle mass for each sex reduced the sex differences in muscle activity per kilogram of muscle. This suggests that using a percentage of HRR could be an effective method for simulating the muscular system during exercises. Therefore, the normalization process is crucial, as it accounts for variations in muscle size, allowing for a more appropriate comparison of muscle activity levels across individuals and sex.

Our findings did not uncover significant differences between males and females in the overall muscle activity required to achieve the specified HRR target during the HDT3 exercises. This suggests that, at least in the early stages of bed rest, sex did not play a critical role in the muscle activation patterns necessary for achieving specific HRR goals. However, a more nuanced picture emerged when we extended our analysis to HDT12. Females exhibited a noticeable decrease in the EMG AUC during CA and PA exercises, alongside an increase in the AUC during HIIT sessions.

The $\dot{V}O_2$peak is a key indicator of aerobic fitness and represents an individual's maximum capacity to uptake, transport, and use oxygen during incremental exercise. There were no significant changes in both male and female exercise participants before and after bed rest, with mean values (± standard error) reported as (36±2.5 and 35.5±3.1 ml/kg/min) for males and (28.7±2.6 and 29.4±2.5 ml/kg/min) for females before and after HDBR, respectively [85, 86]. In contrast, a decrease was observed in the control group participants. This stability in $\dot{V}O_2$peak suggests that the aerobic capacity, and by extension, the ability of the cardiovascular system to deliver oxygen to exercising muscles, was not adversely affected by bed rest, thereby not inducing a central limitation to exercise capacity. Therefore, the increase in EMG signals, especially noted during HIIT sessions, is unlikely to be attributed to central limitations (e.g., changes in cardiac output). Instead, this phenomenon may be more accurately explained by factors related to the peripheral aspects of muscle fatigue, such as the metabolic and mechanical demands placed on muscle fibers during high-intensity exercise, which require increased recruitment of motor units and elevated firing rates to sustain exercise intensity [224, 225].

The contrasting EMG responses observed in females during CA and PA exercises versus HIIT can be further explained by the exercise duration and intensity. CA and PA exercises, with their relatively short duration of 15 minutes, may not induce significant fatigue in muscle fibers, thus not requiring extensive recruitment of additional motor units or heightened firing rates. HIIT, characterized by its prolonged duration of approximately 30 minutes and elevated intensity, is more likely to induce muscle fatigue. This would necessitate greater compensatory muscle activation to sustain the required exercise intensity, as reflected in the increased EMG signal amplitude observed in females during both high-intensity intervals and rest periods on HDT12.

Sex-specific responses further complicate this relationship, with males displaying a more pronounced inflammatory reaction to bed rest, potentially affecting TNF-a levels more significantly and impacting muscle activation and strength outcomes. Although exercise modifies biomarker changes (such as IGF-1, TNF-α, GFAP, UCH-L1, and NfL) suggesting potential neural and inflammatory benefits [169], the connection between these biomarker shifts and baroreflex efficacy remains elusive. This gap underscores the critical need for further exploration into how specific exercise regimens can effectively counter the adverse effects of bed rest on the cardio-postural system by not only modulating biomarker profiles but also enhancing baroreflex sensitivity and function in a sex-specific manner. In addition, understanding sex-related physiological differences is crucial for designing effective training programs for each sex by optimizing performance and health outcomes. For example, training regimens for females could focus on leveraging their higher proportion of Type I fibers by emphasizing endurance and aerobic capacity. In contrast, males might benefit more from strength- and power-oriented training that targets the optimization of Type II muscle fibers.

5.4.2 Cardio-muscular Training Stimulus

Our analysis revealed variations in the EMG AUC values across CA, PA, and HIIT exercises. Participants had specific HRR targets set for the exercise sessions during HDBR. These goals were met on our measurement days (HDT3 and HDT12) without significant differences in sex or time that could have influenced muscle activation outcomes. This uniformity in achieving targeted HRR levels suggests a consistent application and effectiveness of the exercise protocols.

During CA exercise sessions, our target percentage HRR was around 60-70%, comparable to the max intensity window in PA. This shows that the maximum cardiovascular stresses are similar in both exercises, despite PA having a lower average value than CA over the entire test. However, we observed a higher EMG AUC during PA than during CA (see Figure 5.2). This can be attributed to several factors: (1) the dynamic intensity of PA stimulates more muscle fibers, potentially increasing EMG

AUC; (2) as PA intensity ramps up, the recruitment of different muscle fibers, especially type II fibers known for significant electrical activity, leads to higher EMG AUC; (3) during CA, the body becomes efficient at performing the same work with a reduced effort over time, whereas PA continually challenges the body to adapt to increasing demands, resulting in greater muscle activation; and (4) the varying stimulus in PA could enhance participant engagement and effort.

Differences in EMG AUC among exercises are also due to varying intensity and duration. HIIT extends approximately 15 minutes longer than CA and PA, involving alternating periods of intense exertion and recovery. This longer duration and intense structure engages more muscle groups, requiring greater energy expenditure and leading to higher muscle activation, as shown in Figure **5.2**. HIIT's effectiveness includes increased oxygen consumption ($\dot{V}$O2) compared with steady-state exercises. This reflects the body's heightened metabolic needs during high-intensity intervals and recovery periods, contributing to a greater training stimulus. Essentially, CA and PA push your heart similarly, but PA activates more muscles because of its dynamic nature. HIIT, being more intense and longer, engages more muscles and requires more energy, leading to higher muscle activation and better overall fitness. HIIT also makes your body consume more oxygen, working harder to meet energy needs and providing a stronger training effect.

These results showed a reduction in EMG AUC and an increased standard deviation on HDT 12 compared with HDT 3 across all exercise modalities (CA, PA, and HIIT). These findings are most likely the product of several physiological adaptations and responses to prolonged inactivity and bed rest. First, prolonged bed rest induces muscle atrophy, particularly in the postural muscles, leading to a loss of muscle mass and strength. Second, neural adaptations, characterized by downregulated neural drive to muscles because of lack of movement and reduced gravitational forces, result in fewer or less frequent motor unit recruitments, reducing EMG readings. Metabolic shifts from aerobic to anaerobic pathways can diminish muscle efficiency and activation, and psychological factors, such as decreased motivation and mental fatigue, may further influence voluntary muscle activation. Finally, compensatory mechanisms may emerge, with weaker muscles altering activation patterns and other muscles compensating, leading to increased variability in data as individuals adopt different strategies to perform tasks. Collectively, these factors contribute to the observed reduction in EMG AUC and increased standard deviation after prolonged bed rest.

5.4.3 Time-Dependent Responses in Exercises

No significant changes in muscle activity were observed between HDT3 and HDT12 for CA, PA, and HIIT. This may demonstrate the preservation of muscle activation by exercise countermeasures. However, our previous analysis of these data [34] showed that 14 days of HDBR significantly reduced the muscle pump baroreflex system in all groups, regardless of sex or exercise.

One potential explanation for the ineffectiveness of the proposed exercise countermeasures in this bed rest study is the choice of exercising in a 6º HDT position without an orthostatic stressor. The baroreflex mechanism is essential for maintaining cardiovascular stability, regulating heart rate, muscle-pump activation, and vasoconstriction to respond to fluctuations in blood pressure, and ensuring that blood pressure remains within a normal range. Specifically, when blood pressure rises, the baroreflex mechanism typically responds by decreasing heart rate and vasodilating to lower blood pressure; conversely, if blood pressure drops, it increases heart rate and vasoconstriction to elevate blood pressure. During exercise, muscle contractions assist in venous return. This is important during upright exercise because it counteracts gravitational venous pooling and enhances cardiac output.

During exercises performed in an upright position, the baroreflex system stabilizes blood pressure against heart rate increases and vascular changes induced by exercise, ensuring that blood pressure fluctuations remain within safe limits. This equilibrium is achieved through a complex interplay of cardiovascular responses, including the muscle pump baroreflex, which responds to changes in body posture and gravity. However, exercises conducted in the HDT, or the supine position, bypass the gravitational cues essential for the activation of the muscle pump baroreflex. Unlike upright positions that impose orthostatic stress and stimulate the baroreflex system to adjust blood pressure in response to gravitational shifts, HDT and supine positions do not mimic these natural standing or vertical stresses. Consequently, these HDT exercise modalities cannot effectively engage the muscle pump baroreflex mechanism.

Therefore, the absence of this gravitational challenge during 6º HDT exercise could explain why the exercise interventions did not produce the expected outcomes in terms of baroreflex preservation. Without the natural stimulus provided by orthostatic stress, the baroreflex system may not respond as effectively to changes in heart rate and blood pressure induced by exercise. This suggests that 6º HDT or supine positions may not be the most conducive for exercises aimed at enhancing or preserving baroreflex sensitivity and function.

5.4.4 Limitations and Future Directions

The results of our study provided a detailed examination of certain shortcomings associated with intervention exercises, specifically when analyzed by sex. This study shows a nuanced approach to understanding the effectiveness or limitations of the intervention in the context of sex differences. This approach allows for a more targeted and detailed exploration of how the exercises may have different effects or encounter specific challenges when applied to male and female astronauts or patients in bed rest. Ideally, to optimize protocols and countermeasure equipment, we should personalize the ideal combination of elements—such as modality, intensity, duration, and frequency of exercises—and target specific muscle groups for each individual, ensuring success in the unique space environment.

Given the limitations observed with HIIT and aerobic exercise modalities in fully engaging male lower leg muscles, our results suggest that incorporating alternative types of exercise could be beneficial. Resistance training, for example, might offer distinct physiological stimuli that could enhance baroreflex sensitivity and muscle activation differently than aerobic or HIIT sessions. Baroreflex preservation is crucial for regulating blood pressure during orthostatic challenges in a gravitational field, which can directly impact the success of future space missions during lunar or Martian landings. Therefore, exploring the inclusion of high intensity resistance exercises could provide a more comprehensive approach to exercise programing, potentially improving outcomes for male participants in terms of muscle activation and baroreflex function. Furthermore, lower body negative pressure (LBNP) devices, or centrifuge artificial gravity, can provide the required force to direct a greater volume of blood to the body's lower extremities, helping to maintain blood flow and pressure regulation. This can be useful for baroreflex training, as it directly targets the body's ability to regulate blood pressure in conditions that mimic standing on Earth.

As one of the eight research groups integrated into the HDBR project, our study concentrated on the decline in cardio-postural control during bed rest and how exercise countermeasures can blunt this reduction, with numerous measurements extending beyond our study's primary focus. An external expert committee [187] developed the multimodal exercise intervention, which underpins the framework for all funded research programs. This comprehensive approach offers a structured context for our current observations in baroreflex characteristics, though it presents challenges in directly attributing these observations to specific exercises. As we move forward, both our team and other groups involved in the Canadian aging and inactivity study plan to conduct detailed analyses to explore these aspects further. Integrating centrifugation or LBNP techniques into our countermeasures for physiological preservation during space missions could enhance the effectiveness of our interventions.

5.5 Conclusion

The decline in cardio-postural function and muscular performance with aging or inactivity highlights the critical need for effective exercise countermeasures. Our exploration of the effects of space-based exercise interventions on cardio-postural control and muscle attributes within the Canadian aging and inactivity study provides valuable insights into designing effective exercise countermeasures, particularly for older adults and for conditions simulating spaceflight. Our findings emphasize the need to reevaluate exercise modalities, giving priority to upright positions and activities that simulate daily orthostatic challenges. The aim is to engage the baroreflex system more effectively and maintain cardiovascular health. During bed rest, if we do not include orthostatic stressors, even while performing exercises, we will not activate the baroreflexes. The cardio-postural system will lose its functionality because of adaptation to the new environment. The observed sex-specific discrepancies in muscle activation and training impulse emphasize the necessity of sex-specific training programs that optimize performance and health outcomes, paving the way for a more inclusive and effective strategy in combating the adverse effects of aging and inactivity on the human body.

Chapter 6. Conclusions and Future Directions

6.1 Conclusions

Regulating arterial blood pressure and maintaining postural stability are essential for maintaining an upright posture. To maintain blood pressure while standing, the autonomic nervous system activates the arterial baroreflex through efferent neural pathways, leading to an increase in heart rate, systemic vascular resistance, and cardiac contractility. However, regulating blood pressure while standing can be challenging. Calf (gastrocnemius, soleus) and tibialis anterior contractions are required to maintain an upright posture by keeping the center of mass within the base of support [55, 57, 58]. Moreover, lower limb muscle contractions result in the constriction of the underlying veins, which leads to the pumping of pooled venous blood back to the heart (i.e., skeletal muscle pump), resulting in increased venous return and blood pressure [35, 226]. Therefore, blood pressure and postural regulation during standing require continuous feedback from the cardiovascular, postural, and musculoskeletal systems. However, spaceflight and bed rest both induce cardiovascular and musculoskeletal changes, leading to an increase in the risk of orthostatic intolerance (OI). Mechanisms contributing to OI after spaceflight or bed rest are multifaceted, including failure to maintain arterial blood pressure because of changes in baroreflex function (cardiac, vasoconstriction, muscle-pump) coupled with reduced blood volume and venous return [17, 227, 228] or inadequate arterial vasoconstriction [229, 230].

This thesis has made contributions toward the assessment of the effects of 14 days of 6-degree HDBR with or without combined lower body strength, aerobic, and HIIT exercise countermeasures to understand the role of lower limb muscle contractions in maintaining blood pressure and postural stability in 55- to 65-year-old adults while standing before and after HDBR. Below are the principal findings and conclusions.

6.2 Cardio-Postural Coupling in Older Adults During Orthostatic Challenge

Our findings revealed significant changes, including reduced SBP, EMG, and EMGimp, coupled with heightened heart rate, venous blood pooling, and postural sway upon standing after bed rest. We found that older adults following HDBR have impaired cardiac baroreflex and reduced mechanical effect of the heart on blood pressure during standing. In addition, older adults demonstrated muscle-pump baroreflex impairment following HDBR along with mechanical muscle pump dysfunction, which could lead to a drop in BP. The cardiac baroreflex had a limited effect on blood pressure during standing, whereas lower limb muscles continued to contract and maintain blood pressure via the muscle-pump mechanism. In addition, physical inactivity through bed rest reduced both cardiac and muscle-pump baroreflex activation (reduced gain and FTA) during a free-standing orthostatic challenge.

In this first Canadian aging and inactivity study (CAIS), the exercise intervention of upper and lower body strength, aerobic, and HIIT exercise countermeasures did not impact the decline in cardiac baroreflex and only partially achieved success in preserving the muscle-pump baroreflex, despite significant preservation of beat-to-beat muscle activity during standing. Further analysis is necessary to expand our understanding of the neural coupling involved in the interaction between muscle activation during exercise and the blood pressure reflex.

6.3 Contribution of Individual Lower Leg Muscles to the Muscle Pump Baroreflex During Orthostatic Stress

We explored the effect of 14 days of HDBR on the muscle-pump baroreflex of distinctive lower leg muscle groups among 55- to 65-year-old men and women. We showed that the cardio-postural system impairment of the individual leg muscles of older adults after HDBR was associated with sex and intervention. Following HDBR, male and female controls experienced the most pronounced decline in muscle-pump causality and postural stability, respectively, compared with the exercise groups. Our results revealed differing strategies between males and females to counteract these impairments.

Postural stability was predominantly compromised in the female control group, as indicated by increased COPr and COPrv values. This could be attributed to a decrease in the overall EMG while standing. The diminished muscle engagement likely resulted in a longer response time for correcting any disturbances in body motion. Our results suggested that the elevated postural sway in the female control group on R+0 led to greater involvement of lower leg muscles in BP regulation during standing, as shown by a notable increase in the SBP → COPr segment in the cardio-postural system, along with a considerable decline in SBP → EMGimp.

Muscle activation induced by postural sway varied among the corresponding leg muscles. Males and females on BDC-1 did not differ in the CoP→EMGimp causality between the respective leg muscles. However, on R+0, the TA muscle had a significant increase in the CoP→EMGimp causality compared with LG, MG, and SOL in females, whereas males did not show a considerable difference between the corresponding leg muscles. These differences in posture-induced muscle activity in the TA, but not in other leg muscles, could account for the R+0 increase in EMGimp seen in female subjects, which hypothetically led to greater involvement of the TA muscle during standing.

Males in the control group, but not in the exercise group, exhibited a significant decrease in the activity of LG, SOL, and TA as measured by EMG and EMGimp on R+0. This shows that the proposed exercise intervention may have the potential to preserve these muscles following HDBR. There was a smaller decrease in both overall normalized EMG and EMGimp based on muscle mass in females, indicating that males are more susceptible to muscle atrophy following a period of inactivity. Variations in muscle mass and the distinct composition of fiber types in each sex may explain the differences in muscle atrophy between males and females following HDBR. The varied responses of lower leg muscles in males and females to mitigate the effects of HDBR emphasize the significance of customizing exercise interventions according to sex.

This alteration contributes to an augmentation in venous return, improving blood flow back to the heart, which impacts cardiac output and consequently influences blood pressure. This phenomenon highlights the intricate interplay between postural stability, muscular activity, and cardiovascular regulation in older female adults, shedding light on potential strategies for mitigating postural instability-related risks, such as falls and orthostatic intolerance.

6.4 Effects of Aerobic and High-Intensity Interval Training on Muscle Pump Baroreflex preservation in Older Adults After Bed rest

In our study, we observed significant sex differences in muscle activation during exercise, with females displaying lower muscle activity per kilogram of muscle mass than males, yet achieving similar TRIMP, indicating a potentially greater training stimulus under similar conditions. Despite these sex-specific responses, our findings suggest that the exercise countermeasures employed, while beneficial for overall cardiovascular health and muscle tone, may not fully protect the muscle-pump baroreflex system after prolonged bed rest. This highlights a crucial gap in current exercise countermeasures, underscoring the need for innovative solutions that can effectively simulate orthostatic stress or gravitational forces to preserve baroreflex sensitivity during extended periods of microgravity exposure or bed rest.

The investigation into responses to exercise revealed that the prescribed countermeasures did not result in significant changes in muscle activity over the course of the bed rest study, suggesting an inability to fully preserve the muscle-pump baroreflex system. The 6º HDT position adopted during exercise may limit the effectiveness of these countermeasures by failing to provide the orthostatic stress required for the normal functioning of the baroreflex system. This underlines the importance of rethinking exercise protocols in simulated microgravity environments to ensure that they include components that mimic gravitational challenges, enhancing the preservation of cardiovascular regulatory mechanisms.

Finally, our study also delved into the biomechanical and anatomical differences between sexes, revealing that these variations do not significantly impact the overall muscle activity required to achieve specific Heart Rate Reserve (HRR) targets during the early stages of bed rest. However, a nuanced analysis showed that females exhibited distinct EMG responses to the different exercises, with an increase in muscle activation during HIIT. This suggests that exercise intensity and duration play critical roles in inducing muscle fatigue, necessitating a sex-specific approach to training programs to optimize performance and health outcomes.

6.5 Space Exploration Applications

While there are parallels between post-bed rest and post-spaceflight data, it is important to exercise caution when extrapolating bed rest data to predict cardio-postural system responses during planetary exploration on the Moon (1/6 G) and Mars (3/8 G). It is reasonable to expect that most bed rest responses to supine exercise will mirror exercise responses in microgravity, and post-bed rest standing may serve as an effective model for immediate postflight orthostatic responses upon return to Earth [231]. However, it is important to note that orthostatic intolerance in partial gravity, specifically on the Moon and Mars, has not been examined.

Currently, determining the precise exercise conditioning and level of physical fitness necessary for performing extravehicular activities (EVA) on the Moon and Mars remains challenging. It appears reasonable to expect that partial gravity will impose smaller orthostatic challenges on astronauts when standing after microgravity exposure compared with the challenges experienced upon returning to the full gravity of Earth. However, unlike the scenario of returning to Earth, astronauts landing on the Moon and Mars will be expected to engage autonomously in construction and exploration activities immediately, without the luxury of a one to two-month reconditioning program [232, 233]. Consequently, the development of countermeasures to safeguard the cardio-postural system during prolonged microgravity transit (approximately 180 days to Mars) may prove crucial for the successful execution of planetary EVA soon after arriving on the Martian surface.

Testing countermeasures during bed rest, particularly in the 6º HDT position, provides the most relevant data for addressing astronauts' physiological deconditioning during extended space flights. From a physiological perspective, in-bed exercise during space missions serves as a "counteract mode," where astronauts are required to exercise to preserve the functionality of the cardio-postural system during the period of readjustment to a gravitational field. However, identifying the optimal exercise countermeasure for spaceflight remains a challenge, and further investigations are necessary to clarify this aspect.

6.6 Applications on Earth

The rapid increase in the number of older individuals within global populations presents an unprecedented set of challenges. Normal aging is accompanied by physical changes, such as diminished tonic vagal control of the heart, reduced baroreflex control of heart rate when standing, and a decline in both mass and quality of skeletal muscle, all of which contribute to the predisposition for orthostatic intolerance [234, 235]. Older adults on Earth live in a typical 1g environment but exhibit physiological dysfunctions. In contrast, astronauts live in an atypical environment (0g) with regular physiology. This unique environment induces distinctive physiological changes, leading to orthostatic intolerance upon landing on a gravitational field after months of microgravity exposure. Despite the unique characteristics of space, there are notable similarities between spaceflight, aging, and physical inactivity in their potential to induce OI [236].

The experiences of astronauts serve as a reminder that the autonomic nervous system's ability to adapt to terrestrial gravity cannot be taken for granted. Orthostatic intolerance, manifesting in various forms, is a prevalent clinical challenge on Earth [237, 238]. Broadly, orthostatic intolerance syndromes can be categorized into neurally mediated syncope, postural tachycardia syndrome (POTS), and orthostatic hypotension. Neurally mediated syncope ranks among the most common reasons for emergency room visits [239]. Patients with neurally mediated syncope exhibit normal cardiovascular autonomic control until triggered by factors like prolonged standing, leading to hypotension with or without bradycardia. As suggested by its name, POTS is characterized by an exaggerated heart rate response and hyperadrenergic symptoms upon standing [240]. POTS is among the most prevalent autonomic nervous system disorders [241], primarily affecting younger women, but not exclusively. Conversely, orthostatic hypotension, marked by sustained reductions in blood pressure upon standing, becomes more prevalent with advancing age [242].

Although space research contributes to our understanding of clinical applications, attributing all space-induced physiological changes to clinical conditions is challenging. For instance, orthostatic intolerance following actual or simulated spaceflight exhibits similarities to neurally mediated syncope or postural tachycardia syndrome (POTS) rather than the immediate and sustained reduction in blood pressure observed in patients with orthostatic hypotension. A single mechanism cannot explain orthostatic intolerance following spaceflight. Individual predisposition, volume loss, cardiovascular deconditioning, autonomic nervous system adaptation, skeletal muscle atrophy, reduced muscle pump baroreflex, and, potentially, changes in cerebral autoregulation may collectively contribute to a negative impact on orthostatic tolerance. Although the various orthostatic intolerance syndromes encountered in clinical settings have distinct underlying causes, the observation that multiple factors influence orthostatic tolerance holds clinical relevance. For instance, hypovolemia exacerbates all these conditions. Conversely, measures that mitigate venous pooling may offer improvements in neurally mediated syncope, POTS, and orthostatic hypotension.

The direct application of spaceflight countermeasures to older bed-confined individuals may be impractical because of their limitations in performing intense exercises. However, the foundational countermeasure principles can be adapted for broader use in therapeutic programs catering to older adults and patients with limited mobility. Assessing and understanding the impact of space-based countermeasures offers an opportunity to recalibrate exercise intensity, target specific muscle groups, incorporate rehabilitation principles, embrace a holistic approach addressing both physical and psychological aspects, tailor interventions based on individual health conditions, leverage advanced technologies, and collaborate with healthcare professionals. This allows for the development of a customized and innovative strategy aimed at enhancing the health and well-being of older individuals and patients. This adaptive approach ensures that the fundamental principles of spaceflight countermeasures effectively contribute to maintaining health in populations facing mobility challenges.

Despite the parallels and distinctions between aging and spaceflight, continuous research efforts are imperative. Physiological changes during spaceflight occur at a faster pace than aging, and the unique conditions of spaceflight, with its simultaneous influence on multiple physiological processes, amplify the potential for highly synergistic effects. Furthermore, investigating the primary causes of OI is complicated by alterations in hormones, activity levels, nutrition, and disease, which are intertwined with age-related physiological changes. It is hoped that the engineering expertise continually pushed to its limits in space research will yield new treatments for patients with OI on Earth.

6.7 Recommended Guidelines for the Creation of OI Countermeasures for EVA

As NASA envisions long-duration human missions lasting up to 1100 days in space, the quest for developing countermeasures to maintain astronauts' health and performance remains ongoing. Despite several decades of investigation, the challenge of OI during a Lunar or Mars EVA persists. The optimal countermeasure strategy for preventing spaceflight-induced deconditioning would involve a treatment approach that preserves each organ system's condition similar to that in a normal gravity environment while minimizing crew time and vehicle resource commitments. Countermeasures that are excessively time-consuming, intense, or complex may hinder crew compliance and diminish overall effectiveness [243]. Although bed rest serves as a well-controlled model for testing and refining countermeasure hardware and protocols, successful countermeasure implementation in spaceflight missions requires consideration of the logistical implications of the spaceflight environment. This includes factors such as crew schedules, hardware mass, volume, stowage, and the impact of the countermeasure on environmental control systems.

Although studies have validated that a combination of resistive and aerobic exercise training programs during bed rest or spaceflight can mitigate losses in muscle strength and endurance, as well as lean leg mass, for all sexes [244, 245], it is noteworthy that women often have lower muscle strength and cardiovascular endurance than men [246, 247]. This discrepancy may elevate the risk of OI after spaceflight for women, despite the implementation of intensive exercise countermeasures. For instance, in a recent bed rest study led by Lee et al., indicators of muscle strength and endurance in female subjects were at the lower end of the spectrum seen among ISS astronauts [244]. To address this discrepancy, personalized exercise routines targeting improvements in cardiac volume and mass, both of which have been shown to enhance orthostatic tolerance, have been recommended [244, 248]. The results of our study show that to maintain orthostatic tolerance after space missions, exercise countermeasures need to focus on specific muscles, i.e., the TA in females and the MG in males.

Effective exercise countermeasures for less-fit individuals, as determined before bed rest or spaceflight, may involve high-intensity exertion exercises to maintain intramuscular pressure. Meanwhile, for more fit individuals, tailored frequency and periodization regimes involving variations in volume and intensity are considered more beneficial [244]. Significant sex differences have also been noted in muscular strength across different body regions, necessitating careful consideration when designing tailored exercise programs [247].

While countermeasures are typically evaluated on the basis of their ability to sustain preflight performance levels, exploration mission crew members may be required to perform certain tasks (under normal or emergency situations) that are not ‘scaled’ to preflight fitness levels. For instance, maintaining trunk and lower-body musculature is crucial when landing on an extraterrestrial surface, as no support personnel will be available to assist the crew in egressing the vehicle [244, 249, 250]. A previous study assessed 13 Space Shuttle astronauts before their mission, and two had insufficient fitness levels to complete a simulated emergency egress [251]. Therefore, concentrating on the effectiveness of exercise countermeasures to maintain preflight performance levels may not be the most appropriate benchmark in certain circumstances. Tailoring exercise regimes to reduce the risk of OI while astronauts perform various exploration mission-related responsibilities, including physically demanding tasks under time constraints, should be a priority for future research [249].

6.8 Future Directions

The methods developed in this thesis and with novel results on the mechanisms involved in blood pressure and postural control in an older population led to future work for the development of newer methods and clinical insights. Below are some suggestions for future work.

Large cohort analysis: Future work with larger cohorts is needed to assess cardio-postural, cardio-respiratory, and posture synchronization to fully confirm the effect of bed rest and inactivity on these systems during standing. This will also aid in the development of generalized outcomes and intervention strategies to prevent OH and associated falls in older adults.

Cardiorespiratory interaction assessment: Respiration affects postural balance through the movement of the diaphragm and ribcage, which mechanically displaces the COM and causes postural perturbations. Moreover, respiration can stimulate the cerebral cortex and alter lower and upper extremity muscle control and postural balance. In addition, hyperventilation reduces blood carbon dioxide content, leading to increased cerebral vasculature constriction, dizziness, and postural instability, whereas lower limb muscle activation compensates for respiratory-induced postural perturbations. Therefore, a thorough understanding of the effect of respiration on blood pressure, postural control, and lower limb muscle activation is necessary to understand the physiology of postural control in older adults following HDBR. Such knowledge can aid in the development of measures to prevent falls in older adults.

Older adults with disease: The older adults studied in this research were healthy; therefore, the role of interactions between the cardiovascular, respiratory, musculoskeletal, and postural systems in blood pressure regulation and postural control should be explored in advanced stages of diseases, such as in patients with stroke, diabetes mellitus, Parkinson’s disease, Alzheimer’s disease, spinal cord injury, multiple sclerosis, and traumatic brain injury.

Include data from other muscles: Hip and trunk muscles affect respiration and postural control. Therefore, it would be interesting to investigate the relative contribution of these muscles to breathing and postural sway in older adults following HDBR. Investigation of the effect of abdominal effort on lower limb muscles, blood pressure, and postural sway would help in understanding posturo-synchronization, blood pressure, and postural control systems.

Blood pooling measurement: blood pooling during standing was not measured. Therefore, quantitative measurement of blood pooling in the lower limbs during an orthostatic challenge will provide more information about the behavior of the cardio-postural model in older adults.

References

1. Goswami, N., et al., *Using the Moon as a high-fidelity analogue environment to study biological and behavioral effects of long-duration space exploration.* Planetary and Space Science, 2012. **74**(1): p. 111-120.
2. Hedge, E.T., et al., *Implementation of exercise countermeasures during spaceflight and microgravity analogue studies: Developing countermeasure protocols for bedrest in older adults (BROA).* Frontiers in Physiology, 2022: p. 1443.
3. Convertino, V.A., et al., *Head-down bed rest impairs vagal baroreflex responses and provokes orthostatic hypotension.* Journal of Applied Physiology, 1990. **68**(4): p. 1458-1464.
4. Convertino, V.A., et al., *Changes in volume, muscle compartment, and compliance of the lower extremities in man following 30 days of exposure to simulated microgravity.* Aviation, space, and environmental medicine, 1989. **60**(7): p. 653-658.
5. LeBlanc, A.D., et al., *Skeletal responses to space flight and the bed rest analog: a review.* Journal of Musculoskeletal and Neuronal Interactions, 2007. **7**(1): p. 33.
6. Platts, S.H., et al., *Cardiovascular adaptations to long-duration head-down bed rest.* Aviation, space, and environmental medicine, 2009. **80**(5): p. A29-A36.
7. Spector, E.R., S.M. Smith, and J.D. Sibonga, *Skeletal effects of long-duration head-down bed rest.* Aviation, space, and environmental medicine, 2009. **80**(5): p. A23-A28.
8. Mechling, H. and Y. Netz, *Aging and inactivity—capitalizing on the protective effect of planned physical activity in old age.* European Review of Aging and Physical Activity, 2009. **6**(2): p. 89-97.
9. Wang, J., et al., *Neuroprotective effect of several phytochemicals and its potential application in the prevention of neurodegenerative diseases.* Geriatrics, 2016. **1**(4): p. 29.
10. Chodzko-Zajko, W.J., et al., *Exercise and physical activity for older adults.* Medicine & science in sports & exercise, 2009. **41**(7): p. 1510-1530.
11. Lanier, J.B., M.B. Mote, and E.C. Clay, *Evaluation and management of orthostatic hypotension.* American family physician, 2011. **84**(5): p. 527-536.
12. Hargens, A.R. and L. Vico, *Long-duration bed rest as an analog to microgravity.* Journal of applied physiology, 2016. **120**(8): p. 891-903.
13. Greenleaf, J., et al., *Work capacity during 30 days of bed rest with isotonic and isokinetic exercise training.* Journal of applied physiology, 1989. **67**(5): p. 1820-1826.
14. Stremel, R., et al., *Cardiorespiratory deconditioning with static and dynamic leg exercise during bed rest.* Journal of applied physiology, 1976. **41**(6): p. 905-909.
15. van Duijnhoven, N.T., et al., *Resistive exercise versus resistive vibration exercise to counteract vascular adaptations to bed rest.* Journal of Applied Physiology, 2010. **108**(1): p. 28-33.
16. Kramer, A., et al., *How to prevent the detrimental effects of two months of bed-rest on muscle, bone and cardiovascular system: an RCT.* Scientific reports, 2017. **7**(1): p. 13177.

17. Shibata, S., M. Perhonen, and B.D. Levine, *Supine cycling plus volume loading prevent cardiovascular deconditioning during bed rest.* Journal of applied physiology, 2010. **108**(5): p. 1177-1186.
18. Iwasaki, K.-i., et al., *Hypergravity exercise against bed rest induced changes in cardiac autonomic control.* European journal of applied physiology, 2005. **94**: p. 285-291.
19. Schneider, S.M., et al., *WISE-2005: exercise and nutrition countermeasures for upright VO2pk during bed rest.* Medicine and science in sports and exercise, 2009. **41**(12): p. 2165-2176.
20. Watenpaugh, D.E., et al., *Supine lower body negative pressure exercise during bed rest maintains upright exercise capacity.* Journal of Applied Physiology, 2000. **89**(1): p. 218-227.
21. Coupé, M., et al., *Low-magnitude whole body vibration with resistive exercise as a countermeasure against cardiovascular deconditioning after 60 days of head-down bed rest.* American Journal of Physiology-Regulatory, Integrative and Comparative Physiology, 2011. **301**(6): p. R1748-R1754.
22. Guinet, P., et al., *MNX (medium duration nutrition and resistance-vibration exercise) bed-rest: Effect of resistance vibration exercise alone or combined with whey protein supplementation on cardiovascular system in 21-day head-down bed rest.* Frontiers in Physiology, 2020. **11**: p. 812.
23. Ried-Larsen, M., H.M. Aarts, and M.J. Joyner, *Effects of strict prolonged bed rest on cardiorespiratory fitness: systematic review and meta-analysis.* Journal of Applied Physiology, 2017. **123**(4): p. 790-799.
24. Kovacs, G.T. and M. Shadden, *Analysis of age as a factor in NASA astronaut selection and career landmarks.* PloS one, 2017. **12**(7): p. e0181381.
25. Di Girolamo, F.G., et al., *The aging muscle in experimental bed rest: a systematic review and meta-analysis.* Frontiers in nutrition, 2021: p. 204.
26. Diedrich, A., S.Y. Paranjape, and D. Robertson, *Plasma and blood volume in space.* The American journal of the medical sciences, 2007. **334**(1): p. 80-86.
27. Ivanov, P.C., *The new field of network physiology: building the human physiolome.* Frontiers in Network Physiology, 2021: p. 1.
28. Marinescu, I.E., P.N. Lawlor, and K.P. Kording, *Quasi-experimental causality in neuroscience and behavioural research.* Nature human behaviour, 2018. **2**(12): p. 891-898.
29. Rolls, E.T., *Mind causality: a computational neuroscience approach.* Frontiers in Computational Neuroscience, 2021. **15**: p. 706505.
30. Faes, L., G. Nollo, and A. Porta, *Mechanisms of causal interaction between short-term RR interval and systolic arterial pressure oscillations during orthostatic challenge.* Journal of applied physiology, 2013. **114**(12): p. 1657-1667.
31. Porta, A., et al., *Causal relationships between heart period and systolic arterial pressure during graded head-up tilt.* American Journal of Physiology-Regulatory, Integrative and Comparative Physiology, 2011. **300**(2): p. R378-R386.
32. Młyńczak, M. and H. Krysztofiak, *Discovery of causal paths in cardiorespiratory parameters: a time-independent approach in elite athletes.* Frontiers in physiology, 2018. **9**: p. 1455.

33. Porta, A., et al., *Accounting for respiration is necessary to reliably infer Granger causality from cardiovascular variability series.* IEEE Transactions on Biomedical Engineering, 2011. **59**(3): p. 832-841.
34. Sadeghian, F., et al., *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.* Frontiers in Physiology, 2022: p. 1887.
35. Verma, A.K., et al., *Skeletal muscle pump drives control of cardiovascular and postural systems.* Scientific reports, 2017. **7**(1): p. 45301.
36. Xu, D., et al., *Cardio-postural interactions and muscle-pump baroreflex are severely impacted by 60-day bedrest immobilization.* Scientific Reports, 2020. **10**(1): p. 1-13.
37. Garg, A., et al., *Physiological interdependence of the cardiovascular and postural control systems under orthostatic stress.* American journal of physiology-heart and circulatory physiology, 2014. **307**(2): p. H259-H264.
38. Verma, A.K., et al., *Effect of aging on muscle-pump baroreflex of individual leg muscles during standing.* Frontiers in Physiology, 2019. **10**: p. 845.
39. Xu, D., et al., *Integrative Cardiovascular Physiology and Pathophysiology: Significant role of the cardiopostural interaction in blood pressure regulation during standing.* American Journal of Physiology-Heart and Circulatory Physiology, 2017. **313**(3): p. H568.
40. Tian, F., et al., *Wavelet coherence analysis of dynamic cerebral autoregulation in neonatal hypoxic–ischemic encephalopathy.* NeuroImage: Clinical, 2016. **11**: p. 124-132.
41. Ali, A., et al., *Management of orthostatic hypotension: a literature review.* Cureus, 2018. **10**(8).
42. Ricci, F., R. De Caterina, and A. Fedorowski, *Orthostatic hypotension: epidemiology, prognosis, and treatment.* Journal of the American College of Cardiology, 2015. **66**(7): p. 848-860.
43. Mar, P.L. and S.R. Raj, *Orthostatic hypotension for the cardiologist.* Current opinion in cardiology, 2018. **33**(1): p. 66.
44. Metzler, M., et al., *Neurogenic orthostatic hypotension: pathophysiology, evaluation, and management.* Journal of neurology, 2013. **260**: p. 2212-2219.
45. Lanfranchi, P.A. and V.K. Somers, *Arterial baroreflex function and cardiovascular variability: interactions and implications.* American Journal of Physiology-Regulatory, Integrative and Comparative Physiology, 2002. **283**(4): p. R815-R826.
46. Lohmeier, T.E. and R. Iliescu, *The baroreflex as a long-term controller of arterial pressure.* Physiology, 2015. **30**(2): p. 148-158.
47. Wehrwein, E.A. and M.J. Joyner, *Regulation of blood pressure by the arterial baroreflex and autonomic nervous system.* Handbook of clinical neurology, 2013. **117**: p. 89-102.
48. Benarroch, E.E., *The arterial baroreflex: functional organization and involvement in neurologic disease.* Neurology, 2008. **71**(21): p. 1733-1738.
49. FUXE, K. and G. SEDVALL, *The distribution of adrenergic nerve fibres to the blood vessels in skeletal muscle.* Acta Physiologica Scandinavica, 1965. **64**(1-2): p. 75-86.

50. Samueloff, S.L., N. Browse, and J.T. Shepherd, *Response of capacity vessels in human limbs to head-up tilt and suction on lower body.* Journal of Applied Physiology, 1966. **21**(1): p. 47-54.
51. Magder, S., *Venous mechanics of contracting gastrocnemius muscle and the muscle pump theory.* Journal of Applied Physiology, 1995. **79**(6): p. 1930-1935.
52. Ten Harkel, A., J. Van Lieshout, and W. Wieling, *Effects of leg muscle pumping and tensing on orthostatic arterial pressure: a study in normal subjects and patients with autonomic failure.* Clinical Science, 1994. **87**(5): p. 553-558.
53. Xu, D., et al., *Significant role of the cardiopostural interaction in blood pressure regulation during standing.* American Journal of Physiology-Heart and Circulatory Physiology, 2017. **313**(3): p. H568-H577.
54. Degani, A.M., et al., *Postural Behavior in Medicated Parkinson Disease Patients: A Preliminary Study Searching for Indicators to Track Progress.* Journal of Central Nervous System Disease, 2020. **12**: p. 1179573520922645.
55. Nallegowda, M., et al., *Role of sensory input and muscle strength in maintenance of balance, gait, and posture in Parkinson's disease: a pilot study.* American journal of physical medicine & rehabilitation, 2004. **83**(12): p. 898-908.
56. Orawiec, R.B., S.B. Nowak, and P. Tomaszewski, *Postural stability in Parkinson's disease patients' wives and in elderly women leading different lifestyles.* Health Care for Women International, 2019. **40**(10): p. 1070-1083.
57. Di Giulio, I., et al., *The proprioceptive and agonist roles of gastrocnemius, soleus and tibialis anterior muscles in maintaining human upright posture.* The Journal of physiology, 2009. **587**(10): p. 2399-2416.
58. Le Mouel, C. and R. Brette, *Mobility as the purpose of postural control.* Frontiers in computational neuroscience, 2017. **11**: p. 67.
59. Convertino, V.A., et al., *Hyperventilation in response to progressive reduction in central blood volume to near syncope.* Aviation, space, and environmental medicine, 2009. **80**(12): p. 1012-1017.
60. Convertino, V.A., et al., *Optimizing the respiratory pump: harnessing inspiratory resistance to treat systemic hypotension.* Respiratory Care, 2011. **56**(6): p. 846-857.
61. Rodrigues, G.D., et al., *Respiratory pump contributions in cerebrovascular and postural control responses during orthostatic stress in older women.* Respiratory Physiology & Neurobiology, 2020. **275**: p. 103384.
62. Hodges, P.W., et al., *Coexistence of stability and mobility in postural control: evidence from postural compensation for respiration.* Experimental brain research, 2002. **144**: p. 293-302.
63. Kuczyński, M. and M. Wieloch, *Effects of accelerated breathing on postural stability.* Human movement, 2008. **9**(2): p. 107-110.
64. Kuznetsov, N.A. and M.A. Riley, *Effects of breathing on multijoint control of center of mass position during upright stance.* Journal of motor behavior, 2012. **44**(4): p. 241-253.
65. Hernandez, L., J. Manning, and S. Zhang, *Voluntary control of breathing affects center of pressure complexity during static standing in healthy older adults.* Gait & Posture, 2019. **68**: p. 488-493.

66. Kim, S.-H., H.-J. Shin, and H.-Y. Cho, *Impact of Types of Breathing on Static Balance Ability in Healthy Adults.* International Journal of Environmental Research and Public Health, 2022. **19**(3): p. 1205.
67. Sakellari, V., *The effects of sensory inputs and hyperventilation on postural stability in man*. 1996: University of London, University College London (United Kingdom).
68. Sakellari, V., et al., *The effects of hyperventilation on postural control mechanisms.* Brain: a journal of neurology, 1997. **120**(9): p. 1659-1673.
69. Arnold, A.C. and C. Shibao, *Current concepts in orthostatic hypotension management.* Current hypertension reports, 2013. **15**: p. 304-312.
70. Navaratnarajah, A. and S.H. Jackson, *The physiology of ageing.* Medicine, 2017. **45**(1): p. 6-10.
71. Gupta, V. and L.A. Lipsitz, *Orthostatic hypotension in the elderly: diagnosis and treatment.* The American journal of medicine, 2007. **120**(10): p. 841-847.
72. Pastalan, L.A., *The simulation of age-related sensory losses: A new approach to the study of environmental barriers.* Journal of Visual Impairment & Blindness, 1974. **68**(8): p. 356-362.
73. Monahan, K.D. and C.A. Ray, *Vestibulosympathetic reflex during orthostatic challenge in aging humans.* American Journal of Physiology-Regulatory, Integrative and Comparative Physiology, 2002. **283**(5): p. R1027-R1032.
74. Halter, J.B., *Hazzard's geriatric medicine and gerontology*. 2009.
75. E. Claydon, V., et al., *Orthostatic tolerance and blood volumes in Andean high altitude dwellers.* Experimental physiology, 2004. **89**(5): p. 565-571.
76. Dichgans, J. and H. Diener, *The contribution of vestibulo-spinal mechanisms to the maintenance of human upright posture.* Acta oto-laryngologica, 1989. **107**(5-6): p. 338-345.
77. Monahan, K.D., *Effect of aging on baroreflex function in humans.* American Journal of Physiology-Regulatory, Integrative and Comparative Physiology, 2007. **293**(1): p. R3-R12.
78. Hajjar, I., *Postural blood pressure changes and orthostatic hypotension in the elderly patient: impact of antihypertensive medications.* Drugs & aging, 2005. **22**: p. 55-68.
79. Rehman, H.U. and E.A. Masson, *Neuroendocrinology of ageing.* Age and ageing, 2001. **30**(4): p. 279-287.
80. Sugiyama, Y., et al., *Delayed and diminished pressor response to muscle sympathetic nerve activity in the elderly.* Journal of Applied Physiology, 1996. **80**(3): p. 869-875.
81. Isik, A.T. and P. Soysal, *Orthostatic Hypotension in Older Adults*. 2021: Springer.
82. Goswami, N., et al., *Orthostatic intolerance in older persons: etiology and countermeasures.* Frontiers in physiology, 2017. **8**: p. 803.
83. Phillips, P.A., G.P. Hodsman, and C.I. Johnston, *Neuroendocrine mechanisms and cardiovascular homeostasis in the elderly.* Cardiovascular Drugs and Therapy, 1991. **4**: p. 1209-1213.
84. Hedge, E.T., et al., *Implementation of exercise countermeasures during spaceflight and microgravity analogue studies: Developing countermeasure protocols for a bedrest in older adults (BROA).* Frontiers in Physiology, 2022: p. 1443.

85. Hajj-Boutros, G., et al., *Impact of 14 days of bed rest in older adults and an exercise countermeasure on body composition, muscle strength, and cardiovascular function: Canadian space agency standard measures.* Gerontology, 2023. **69**(11): p. 1284-1294.
86. Hajj-Boutros, G., et al., *Myths and Methodologies: Understanding the health impact of head down bedrest for the benefit of older adults and astronauts. Study protocol of the Canadian Bedrest Study.* Experimental Physiology, 2024.
87. Hermens, H.J., et al., *European recommendations for surface electromyography.* Roessingh research and development, 1999. **8**(2): p. 13-54.
88. Blaber, A.P., C.K. Landrock, and P.A. Souvestre, *Cardio-postural deconditioning: a model for post-flight orthostatic intolerance.* Respiratory physiology & neurobiology, 2009. **169**: p. S21-S25.
89. Garg, A., D. Xu, and A.P. Blaber, *Statistical validation of wavelet transform coherence method to assess the transfer of calf muscle activation to blood pressure during quiet standing.* Biomedical engineering online, 2013. **12**(1): p. 1-14.
90. Rodriguez, J., et al., *Poststroke alterations in heart rate variability during orthostatic challenge.* Medicine, 2017. **96**(14).
91. Verma, A.K., et al., *Skeletal muscle pump drives control of cardiovascular and postural systems.* Scientific Reports, 2017. **7**(1): p. 1-8.
92. Redfern, M.S., J.M. Furman, and R.G. Jacob, *Visually induced postural sway in anxiety disorders.* Journal of anxiety disorders, 2007. **21**(5): p. 704-716.
93. Garg, A., D. Xu, and A. Blaber. *Posture muscle relationship with cardiovascular changes under orthostatic challenge*. in *2014 36th Annual International Conference of the IEEE Engineering in Medicine and Biology Society*. 2014. IEEE.
94. Garg, A., et al. *Alteration in cardiovascular and postural control relationship in non-fainting elderly individuals*. in *2014 36th Annual International Conference of the IEEE Engineering in Medicine and Biology Society*. 2014. IEEE.
95. Pan, T., *Pan J., Tompkins WJ.* A real-time qrs detection algorithm, IEEE Transactions on Biomedical Engineering, 1985. **32**(3): p. 230-236.
96. Masani, K., *Popovic MR, Nakazawa K, Kouzaki M, and Nozaki D.* Importance of body sway velocity information in controlling ankle extensor activities during quiet stance. J Neurophysiol, 2003. **90**: p. 3774-3782.
97. Zemková, E., *Author's reply to Paillard T:"Sport-specific balance develops specific postural skills".* Sports Medicine, 2014. **44**: p. 1021-1023.
98. Konrad, P., *The abc of emg.* A practical introduction to kinesiological electromyography, 2005. **1**(2005): p. 30-5.
99. De Boer, R.W. and J.M. Karemaker, *Cross-wavelet time-frequency analysis reveals sympathetic contribution to baroreflex sensitivity as cause of variable phase delay between blood pressure and heart rate.* Frontiers in Neuroscience, 2019. **13**: p. 694.
100. Yin, L., et al., *Continued spread of HIV among injecting drug users in southern Sichuan Province, China.* Harm reduction journal, 2007. **4**: p. 1-7.
101. Torrence, C. and G.P. Compo, *A practical guide to wavelet analysis.* Bulletin of the American Meteorological society, 1998. **79**(1): p. 61-78.

102. Grinsted, A., J.C. Moore, and S. Jevrejeva, *Application of the cross wavelet transform and wavelet coherence to geophysical time series.* Nonlinear processes in geophysics, 2004. **11**(5/6): p. 561-566.
103. Barraquand, F., et al., *Inferring species interactions using Granger causality and convergent cross mapping.* Theoretical Ecology, 2021. **14**(1): p. 87-105.
104. Tsonis, A.A., et al., *Convergent cross mapping: theory and an example.* Advances in nonlinear geosciences, 2018: p. 587-600.
105. Sugihara, G., et al., *Detecting causality in complex ecosystems.* science, 2012. **338**(6106): p. 496-500.
106. Sugihara, G. and R.M. May, *Nonlinear forecasting as a way of distinguishing chaos from measurement error in time series.* Nature, 1990. **344**(6268): p. 734-741.
107. Vandewoude, M.F., et al., *Malnutrition-sarcopenia syndrome: is this the future of nutrition screening and assessment for older adults?* Journal of aging research, 2012. **2012**.
108. Antonutto, G. and P. Di Prampero, *Cardiovascular deconditioning in microgravity: some possible countermeasures.* European journal of applied physiology, 2003. **90**(3): p. 283-291.
109. Goswami, N., et al., *Maximizing information from space data resources: a case for expanding integration across research disciplines.* European journal of applied physiology, 2013. **113**(7): p. 1645-1654.
110. Moore, A.D., et al., *Cardiovascular exercise in the US space program: past, present and future.* Acta astronautica, 2010. **66**(7-8): p. 974-988.
111. Laws, J., et al., *Systematic review of the technical and physiological constraints of the Orion Multi-Purpose Crew Vehicle that affect the capability of astronauts to exercise effectively during spaceflight.* Acta Astronautica, 2020. **170**: p. 665-677.
112. Convertino, V.A., *Exercise as a countermeasure for physiological adaptation to prolonged spaceflight.* Medicine and Science in Sports and Exercise, 1996. **28**(8): p. 999-1014.
113. Swain, P., et al., *Effectiveness of exercise countermeasures for the prevention of musculoskeletal deconditioning in simulated hypogravity: A systematic review.* Acta Astronautica, 2021. **185**: p. 236-243.
114. Sadeghian, F., et al., *Analysis and Simulation of the Effect of Knee Structure on the Condylar Forces.* Journal of Clinical Physiotherapy Research, 2016. **5**(4): p. e20-e20.
115. Clément, G., *Fundamentals of space medicine*. Vol. 23. 2011: Springer Science & Business Media.
116. Blaber, A.P., K.A. Zuj, and N. Goswami, *Cerebrovascular autoregulation: lessons learned from spaceflight research.* European journal of applied physiology, 2013. **113**(8): p. 1909-1917.
117. Kortebein, P., et al., *Effect of 10 days of bed rest on skeletal muscle in healthy older adults.* Jama, 2007. **297**(16): p. 1769-1774.
118. Goswami, N., *Falls and fall-prevention in older persons: geriatrics meets spaceflight!* Frontiers in physiology, 2017. **8**: p. 603.
119. Goswami, N., et al., *Effect of computerized cognitive training with virtual spatial navigation task during bed rest immobilization and recovery on vascular function: a pilot study.* Clinical Interventions in Aging, 2015. **10**: p. 453.

120. Verma, A.K., et al., *Comparison of autonomic control of blood pressure during standing and artificial gravity induced via short-arm human centrifuge.* Frontiers in physiology, 2018. **9**: p. 712.
121. Verma, A.K., et al., *Non-linear heart rate and blood pressure interaction in response to lower-body negative pressure.* Frontiers in physiology, 2017. **8**: p. 767.
122. Sammons, E.L., et al., *Influence of noninvasive peripheral arterial blood pressure measurements on assessment of dynamic cerebral autoregulation.* Journal of applied physiology, 2007.
123. Panerai, R., et al., *Continuous estimates of dynamic cerebral autoregulation: influence of non-invasive arterial blood pressure measurements.* Physiological measurement, 2008. **29**(4): p. 497.
124. Panerai, R.B., et al., *Transient drifts between Finapres and continuous intra-aortic measurements of blood pressure.* Blood Pressure Monitoring, 2007. **12**(6): p. 369-376.
125. Mathur, S., J. Eng, and D. MacIntyre, *Reliability of surface EMG during sustained contractions of the quadriceps.* Journal of Electromyography and Kinesiology, 2005. **15**(1): p. 102-110.
126. Borst, C. and J.M. Karemaker, *Time delays in the human baroreceptor reflex.* Journal of the autonomic nervous system, 1983. **9**(2-3): p. 399-409.
127. Wallin, B.G., D. Burke, and S. Gandevia, *Coupling between variations in strength and baroreflex latency of sympathetic discharges in human muscle nerves.* The Journal of physiology, 1994. **474**(2): p. 331-338.
128. Pan, J. and W.J. Tompkins, *A real-time QRS detection algorithm.* IEEE transactions on biomedical engineering, 1985(3): p. 230-236.
129. Blaber, A.P., N. Goswami, and D. Xu, *Prolonged unloading of the cardiovascular system during bedrest and spaceflight weakens neural coupling between blood pressure and heart rate.* Acta Astronautica, 2022.
130. Kwak, S.K. and J.H. Kim, *Statistical data preparation: management of missing values and outliers.* Korean journal of anesthesiology, 2017. **70**(4): p. 407.
131. Brunner, E., S. Domhof, and F. Langer, *Nonparametric analysis of longitudinal data in factorial experiments*. Vol. 373. 2002: Wiley-Interscience.
132. Team, R.C., *R Development Core Team. R: A language and environment for statistical computing.* Vienna: R Foundation for Statistical Computing, 2011.
133. Blaber, A.P., et al., *Impairment of cerebral blood flow regulation in astronauts with orthostatic intolerance after flight.* Stroke, 2011. **42**(7): p. 1844-1850.
134. Fritsch-Yelle, J.M., et al., *Spaceflight alters autonomic regulation of arterial pressure in humans.* Journal of Applied Physiology, 1994. **77**(4): p. 1776-1783.
135. Gisolf, J., et al., *Orthostatic blood pressure control before and after spaceflight, determined by time-domain baroreflex method.* Journal of Applied Physiology, 2005. **98**(5): p. 1682-1690.
136. Verheyden, B., et al., *Respiratory modulation of cardiovascular rhythms before and after short-duration human spaceflight.* Acta physiologica, 2007. **191**(4): p. 297-308.
137. Hughson, R.L., et al., *Cardiovascular regulation during long-duration spaceflights to the International Space Station.* Journal of Applied Physiology, 2012. **112**(5): p. 719-727.

138. Hirayanagi, K., et al., *Functional changes in autonomic nervous system and baroreceptor reflex induced by 14 days of 6 head-down bed rest.* European journal of applied physiology, 2004. **92**(1): p. 160-167.
139. Iwasaki, K.-I., et al., *Effect of head-down-tilt bed rest and hypovolemia on dynamic regulation of heart rate and blood pressure.* American Journal of Physiology-Regulatory, Integrative and Comparative Physiology, 2000. **279**(6): p. R2189-R2199.
140. Traon, P.-L., et al., *Cardiovascular responses to orthostatic tests after a 42-day head-down bed-rest.* European journal of applied physiology and occupational physiology, 1997. **77**(1): p. 50-59.
141. Feldstein, C. and A.B. Weder, *Orthostatic hypotension: a common, serious and underrecognized problem in hospitalized patients.* Journal of the American Society of Hypertension, 2012. **6**(1): p. 27-39.
142. Veronese, N., et al., *Orthostatic changes in blood pressure and mortality in the elderly: the Pro. VA Study.* American journal of hypertension, 2015. **28**(10): p. 1248-1256.
143. Möstl, S., et al., *Limited Effect of 60-Days Strict Head Down Tilt Bed Rest on Vascular Aging.* Frontiers in physiology, 2021. **12**.
144. Buszko, K., et al., *Hemodynamic response to the head-up tilt test in patients with syncope as a predictor of the test outcome: A meta-analysis approach.* Frontiers in physiology, 2019. **10**: p. 184.
145. Sibonga, J.D., et al., *Evaluating bone loss in ISS astronauts.* Aerospace medicine and human performance, 2015. **86**(12): p. A38-A44.
146. English, K.L., et al., *Isokinetic strength changes following long-duration spaceflight on the ISS.* Aerospace medicine and human performance, 2015. **86**(12): p. A68-A77.
147. Traon, P.-L., et al., *From space to Earth: advances in human physiology from 20 years of bed rest studies (1986–2006).* European journal of applied physiology, 2007. **101**(2): p. 143-194.
148. Rice, J.E., *Orion Spacecraft*. 2019: Mitchell Lane.
149. Ploutz-Snyder, L.L., et al., *Exercise training mitigates multisystem deconditioning during bed rest.* Medicine and science in sports and exercise, 2018. **50**(9): p. 1920.
150. Goetchius, L., et al. *High intensity training during spaceflight: results from the SPRINT study*. in *Proceedings of the NASA human research program investigators' workshop 'human exploration and discovery: the moon, Mars and beyond*. 2019.
151. Scott, J.P., T. Weber, and D.A. Green, *Introduction to the Frontiers research topic: optimization of exercise countermeasures for human space flight–lessons from terrestrial physiology and operational considerations.* Frontiers in physiology, 2019. **10**: p. 173.
152. Tikhonoff, V., et al., *The uncertain effect of menopause on blood pressure.* Journal of human hypertension, 2019. **33**(6): p. 421-428.
153. Izumi, Y., et al., *Effect of age at menopause on blood pressure in postmenopausal women.* American journal of hypertension, 2007. **20**(10): p. 1045-1050.
154. Taddei, S., *Blood pressure through aging and menopause.* Climacteric, 2009. **12**(sup1): p. 36-40.

155. Claydon, V.E. and R. Hainsworth, *Increased postural sway in control subjects with poor orthostatic tolerance.* Journal of the American College of Cardiology, 2005. **46**(7): p. 1309-1313.
156. Speers, R.A., W.H. Paloski, and A.D. Kuo, *Multivariate changes in coordination of postural control following spaceflight.* Journal of biomechanics, 1998. **31**(10): p. 883-889.
157. Cheshire, W.P., *Hypotensive akathisia: autonomic failure associated with leg fidgeting while sitting.* Neurology, 2000. **55**(12): p. 1923-1926.
158. Blain, H., et al., *EUGMS Falls and Fracture Interest Group; International Association of Gerontology and Geriatrics for the European Region (IAGG-ER); European Union of Medical Specialists (EUMS); Fragility Fracture Network (FFN); European Society for Clinical and Economic Aspects of Osteoporosis and Osteoarthritis (ESCEO), and; International Osteoporosis Foundation (IOF). A comprehensive fracture prevention strategy in older adults: the European Union Geriatric Medicine Society (EUGMS) statement.* Aging Clin Exp Res, 2016. **28**(4): p. 797-803.
159. Rapp, K., et al., *Epidemiology of falls in residential aged care.* J. Am. Med. Dir. Assoc, 2012. **13**.
160. Trozic, I., et al., *Postural hemodynamic parameters in older persons have a seasonal dependency.* Zeitschrift für Gerontologie und Geriatrie, 2020. **53**(2): p. 145-155.
161. Schneider, S.M., et al., *Treadmill exercise within lower body negative pressure protects leg lean tissue mass and extensor strength and endurance during bed rest.* Physiological reports, 2016. **4**(15): p. e12892.
162. Goswami, N., et al., *Lower body negative pressure: physiological effects, applications, and implementation.* Physiological reviews, 2019. **99**(1): p. 807-851.
163. Lee, S.-J., et al., *Targeting myostatin/activin A protects against skeletal muscle and bone loss during spaceflight.* Proceedings of the National Academy of Sciences, 2020. **117**(38): p. 23942-23951.
164. Evans, J.M., C.F. Knapp, and N. Goswami, *Artificial gravity as a countermeasure to the cardiovascular deconditioning of spaceflight: gender perspectives.* Frontiers in Physiology, 2018. **9**: p. 716.
165. Fadil, R., et al., *Cardio-respiratory interactions in response to lower-body negative pressure.* Physiological Measurement, 2023. **44**(2): p. 025005.
166. Borghese, N.A. and A. Calvi, *Learning to maintain upright posture: What can be learned using adaptive neural network models?* Adaptive Behavior, 2003. **11**(1): p. 19-35.
167. Fadil, R., et al., *The Effect of Skeletal Muscle-Pump on Blood Pressure and Postural Control in Parkinson's Disease.* Cardiovascular Engineering and Technology, 2023: p. 1-19.
168. Morita, H., C. Abe, and K. Tanaka, *Long-term exposure to microgravity impairs vestibulo-cardiovascular reflex.* Scientific reports, 2016. **6**(1): p. 33405.
169. Blaber, A.P., et al., *Elevated biomarkers of neural injury in older adults following head-down bed rest: links to cardio-postural deconditioning with spaceflight and aging.* Frontiers in Human Neuroscience, 2023. **17**.

170. Mohammadyari, P., G. Gadda, and A. Taibi, *Modelling physiology of haemodynamic adaptation in short-term microgravity exposure and orthostatic stress on Earth.* Scientific reports, 2021. **11**(1): p. 4672.
171. Alessandro, C., A. Sarabadani Tafreshi, and R. Riener, *Cardiovascular responses to leg muscle loading during head-down tilt at rest and after dynamic exercises.* Scientific reports, 2019. **9**(1): p. 2804.
172. Rabineau, J., et al., *Cardiovascular adaptation to simulated microgravity and countermeasure efficacy assessed by ballistocardiography and seismocardiography.* Scientific reports, 2020. **10**(1): p. 17694.
173. Burgeat, M., et al., *Status of vestibular function after prolonged bedrest.* Acta Astronautica, 1981. **8**(9-10): p. 1019-1027.
174. Jarchow, T. and L. Young, *Neurovestibular effects of bed rest and centrifugation.* Journal of Vestibular Research, 2010. **20**(1-2): p. 45-51.
175. Reschke, M.F., et al., *Postural reflexes, balance control, and functional mobility with long-duration head-down bed rest.* Aviation, space, and environmental medicine, 2009. **80**(5): p. A45-A54.
176. Fadil, R., et al., *Effect of parkinson's disease on cardio-postural coupling during orthostatic challenge.* Frontiers in Physiology, 2022. **13**: p. 863877.
177. Sadeghian, F., et al., *Numerical study of patient-specific ankle-foot orthoses for drop foot patients using shape memory alloy.* Medical Engineering & Physics, 2019. **69**: p. 123-133.
178. Sadeghian, F., et al., *Compliant orthoses for repositioning of knee joint based on super-elasticity of shape memory alloys.* Journal of Intelligent Material Systems and Structures, 2018. **29**(15): p. 3136-3150.
179. Sadeghian, F., et al., *Design and Simulation of a patient-specific ankle orthosis using torsional spring.* Iranian Journal of Mechanical Engineering Transactions of ISME, 2021. **23**(1): p. 22-42.
180. Sadeghian, F., et al., *Design and Analysis of a knee-ankle-foot orthosis using torsional spring.* Modares Mechanical Engineering, 2018. **17**(10): p. 185-193.
181. Kwak, S.K. and J.H. Kim, *Statistical data preparation: management of missing values and outliers.* Korean journal of anesthesiology, 2017. **70**(4): p. 407-411.
182. Jeong, S.-M., et al., *Exercise plus volume loading prevents orthostatic intolerance but not reduction in cerebral blood flow velocity after bed rest.* American Journal of Physiology-Heart and Circulatory Physiology, 2012. **302**(2): p. H489-H497.
183. Hajj-Boutros, G., et al., *Impact of 14 days of bed rest in older adults and an exercise countermeasure on body composition, muscle strength and cardiovascular function: Canadian Space Agency standard measures.* Gerontology, 2023.
184. Engelke, K.A., et al., *Application of acute maximal exercise to protect orthostatic tolerance after simulated microgravity.* American Journal of Physiology-Regulatory, Integrative and Comparative Physiology, 1996. **271**(4): p. R837-R847.
185. Pichot, V., et al., *Interval training in elderly men increases both heart rate variability and baroreflex activity.* Clinical Autonomic Research, 2005. **15**: p. 107-115.
186. Hastings, J.L., et al., *Effect of rowing ergometry and oral volume loading on cardiovascular structure and function during bed rest.* Journal of applied physiology, 2012. **112**(10): p. 1735-1743.

187. Hedge, E.T., et al., *Implementation of exercise countermeasures during spaceflight and microgravity analogue studies: developing countermeasure protocols for bedrest in older adults (BROA).* Frontiers in Physiology, 2022. **13**: p. 928313.
188. Gazibara, T., et al., *Circumstances of falls and fall-related injuries among patients with Parkinson's disease in an outpatient setting.* Geriatric nursing, 2014. **35**(5): p. 364-369.
189. Laughton, C.A., et al., *Aging, muscle activity, and balance control: physiologic changes associated with balance impairment.* Gait & posture, 2003. **18**(2): p. 101-108.
190. Vette, A.H., et al., *Ankle muscle co-contractions during quiet standing are associated with decreased postural steadiness in the elderly.* Gait & posture, 2017. **55**: p. 31-36.
191. Mastrandrea, C.J., et al., *High-intensity exercise does not protect against orthostatic intolerance following bedrest in 55-to 65-yr-old men and women.* American Journal of Physiology-Regulatory, Integrative and Comparative Physiology, 2023.
192. Divsalar, D.N., et al., *A spacecraft-compatible combined artificial gravity and exercise (CAGE) system to sustain astronaut health in the next generation of long-term spaceflight.* Journal of Space Safety Engineering, 2022. **9**(4): p. 577-581.
193. Hagio, K., H. Obata, and K. Nakazawa, *Effects of breathing movement on the reduction of postural sway during postural-cognitive dual tasking.* Plos one, 2018. **13**(5): p. e0197385.
194. Howorka, K., et al., *Effects of guided breathing on blood pressure and heart rate variability in hypertensive diabetic patients.* Autonomic Neuroscience, 2013. **179**(1-2): p. 131-137.
195. Nuckowska, M.K., et al., *Impact of slow breathing on the blood pressure and subarachnoid space width oscillations in humans.* Scientific reports, 2019. **9**(1): p. 1-13.
196. Tipton, C.M., *Historical perspective: Origin to recognition.* ACSM's advanced exercise physiology, 2006: p. 11-28.
197. Meck, J.V., et al., *Marked exacerbation of orthostatic intolerance after long-vs. short-duration spaceflight in veteran astronauts.* Psychosomatic medicine, 2001. **63**(6): p. 865-873.
198. Buckey, J.C., *Space physiology*. 2006: Oxford University Press, USA.
199. VA, C., *Exercise and adaptation to microgravity environments.* The Gravitational Environment, 1995.
200. Sawka, M.N. and A.J. Young, *Physiological systems and their responses to conditions of heat and cold.* ACSM's advanced exercise physiology, 2006. **26**: p. 535-563.
201. Fortney, S.M., V.S. Schneider, and J.E. Greenleaf, *The physiology of bed rest.* Handbook of Physiology. Section 4: Environmental Physiology. 3: The Gravitational Environment, 1996. **2**(Chapter 39).
202. Smith, S.M., et al., *Nutrition in space.* Nutrition Today, 1997. **32**(1): p. 6-12.

203. Trappe, T., et al., *Cardiorespiratory responses to physical work during and following 17 days of bed rest and spaceflight.* Journal of applied physiology, 2006. **100**(3): p. 951-957.
204. Murphy, E., *Postural change and active propulsion: Implications for cardio-postural control in older adults*. 2020, New Mexico State University.
205. Manor, B., et al., *Physiological complexity and system adaptability: evidence from postural control dynamics of older adults.* Journal of Applied Physiology, 2010. **109**(6): p. 1786-1791.
206. Sundblad, P. and O. Orlov, editors, *Guidelines for Standardization of Bed Rest Studies in the Spaceflight Context*. 2015.
207. Cassidy, S., et al., *High intensity intermittent exercise improves cardiac structure and function and reduces liver fat in patients with type 2 diabetes: a randomised controlled trial.* Diabetologia, 2016. **59**: p. 56-66.
208. Convertino, V.A., et al., *Effects of acute exercise on attenuated vagal baroreflex function during bed rest.* Aviation, space, and environmental medicine, 1992. **63**(11): p. 999-1003.
209. Cassidy, S., et al., *Unsupervised high-intensity interval training improves glycaemic control but not cardiovascular autonomic function in type 2 diabetes patients: A randomised controlled trial.* Diabetes and Vascular Disease Research, 2019. **16**(1): p. 69-76.
210. Ploutz-Snyder, L.L., et al., *Integrated resistance and aerobic exercise protects fitness during bed rest.* Med Sci Sports Exerc, 2014. **46**(2): p. 358-368.
211. English, K.L., et al., *High intensity training during spaceflight: results from the NASA Sprint Study.* npj Microgravity, 2020. **6**(1): p. 21.
212. Borst, C., *Karemaker JM.* Time delays in the human baroreceptor reflex. J Auton Nerv Syst, 1983. **9**: p. 399-409.
213. BIAGINI, C., *Validation of EMG envelope for the clinical assessment of muscle activity peak during walking.* 2021.
214. Cogliati, M., A. Cudicio, and C. Orizio, *Using force or EMG envelope as feedback signal for motor control system.* Journal of Electromyography and Kinesiology, 2024. **74**: p. 102851.
215. Farrell, P.A., et al., *Plasma lactate accumulation and distance running performance.* Med Sci Sports, 1979. **11**(4): p. 338-44.
216. Coyle, E.F., *Integration of the physiological factors determining endurance performance ability.* Exercise and sport sciences reviews, 1995. **23**: p. 25-63.
217. Taha, T. and S.G. Thomas, *Systems modelling of the relationship between training and performance.* Sports Medicine, 2003. **33**: p. 1061-1073.
218. Jobson, S.A., et al., *The analysis and utilization of cycling training data.* Sports medicine, 2009. **39**: p. 833-844.
219. Borresen, J. and M.I. Lambert, *The quantification of training load, the training response and the effect on performance.* Sports medicine, 2009. **39**: p. 779-795.
220. Clarke, D.C. and P.F. Skiba, *Rationale and resources for teaching the mathematical modeling of athletic training and performance.* Advances in physiology education, 2013.
221. Banister, E.W., *Modeling elite athletic performance.* Physiological testing of elite athletes, 1991. **347**: p. 403-422.

222. Brunner, E., S. Domhof, and F. Langer, *Nonparametric analysis of longitudinal data in factorial experiments.* (No Title), 2002.
223. Team, R.D.C., *R: A language and environment for statistical computing.* (No Title), 2010.
224. Skelly, L.E., et al., *Human skeletal muscle fiber type-specific responses to sprint interval and moderate-intensity continuous exercise: acute and training-induced changes.* Journal of Applied Physiology, 2021. **130**(4): p. 1001-1014.
225. Lamboley, C.R., et al., *Effects of high-intensity intermittent exercise on the contractile properties of human type I and type II skeletal muscle fibers.* Journal of Applied Physiology, 2020. **128**(5): p. 1207-1216.
226. Verma, A.K. and D. Xu, *Effect of aging on muscle-pump baroreflex of individual leg muscles during standing.* Frontiers in Physiology, 2019. **10**: p. 458287.
227. Levine, B.D., et al., *Maximal exercise performance after adaptation to microgravity.* Journal of applied physiology, 1996. **81**(2): p. 686-694.
228. Iaccarino, G., et al., *Ischemic neoangiogenesis enhanced by β2-adrenergic receptor overexpression: a novel role for the endothelial adrenergic system.* Circulation research, 2005. **97**(11): p. 1182-1189.
229. Waters, W.W., M.G. Ziegler, and J.V. Meck, *Postspaceflight orthostatic hypotension occurs mostly in women and is predicted by low vascular resistance.* Journal of Applied Physiology, 2002. **92**(2): p. 586-594.
230. Buckey Jr, J.C., et al., *Orthostatic intolerance after spaceflight.* Journal of Applied Physiology, 1996. **81**(1): p. 7-18.
231. Cowell, S.A., et al., *The exercise and environmental physiology of extravehicular activity.* Aviation, space, and environmental medicine, 2002. **73**(1): p. 54-67.
232. Katuntsev, V.P., Y.Y. Osipov, and S.N. Filipenkov, *Biomedical problems of EVA support during manned space flight to Mars.* Acta Astronautica, 2009. **64**(7-8): p. 682-687.
233. Payne, M.W., D.R. Williams, and G. Trudel, *Space flight rehabilitation.* American journal of physical medicine & rehabilitation, 2007. **86**(7): p. 583-591.
234. Oida, E., et al., *Aging alteration of cardiac vagosympathetic balance assessed through the tone-entropy analysis.* Journals of Gerontology Series A: Biomedical Sciences and Medical Sciences, 1999. **54**(5): p. M219-M224.
235. Bhati, P., et al., *Does resistance training modulate cardiac autonomic control? A systematic review and meta-analysis.* Clinical Autonomic Research, 2019. **29**: p. 75-103.
236. Shelhamer, M., et al., *Selected discoveries from human research in space that are relevant to human health on Earth.* npj Microgravity, 2020. **6**(1): p. 1-5.
237. Low, P.A., *Neurogenic orthostatic hypotension: pathophysiology and diagnosis.* Am J Manag Care, 2015. **21**(13 Suppl): p. s248-57.
238. Freeman, R., et al., *Consensus statement on the definition of orthostatic hypotension, neurally mediated syncope and the postural tachycardia syndrome.* Autonomic Neuroscience, 2011. **161**(1-2): p. 46-48.
239. Mathias, C.J., K. Deguchi, and I. Schatz, *Observations on recurrent syncope and presyncope in 641 patients.* The Lancet, 2001. **357**(9253): p. 348-353.
240. Low, P.A., et al., *Postural tachycardia syndrome (POTS).* Journal of cardiovascular electrophysiology, 2009. **20**(3): p. 352-358.

241. Raj, S.R., et al., *Postural orthostatic tachycardia syndrome (POTS): Priorities for POTS care and research from a 2019 National Institutes of Health Expert Consensus Meeting–Part 2.* Autonomic Neuroscience, 2021. **235**: p. 102836.
242. Schroeder, C., J. Jordan, and H. Kaufmann, *Management of neurogenic orthostatic hypotension in patients with autonomic failure.* Drugs, 2013. **73**: p. 1267-1279.
243. Lee, S., et al., *Supine LBNP exercise maintains exercise capacity in male twins during 30-d bed rest.* Medicine and science in sports and exercise, 2007. **39**(8): p. 1315-1326.
244. Lee, S.M., et al., *WISE-2005: Countermeasures to prevent muscle deconditioning during bed rest in women.* Journal of applied physiology, 2014. **116**(6): p. 654-667.
245. Hackney, K.J., et al., *The astronaut-athlete: optimizing human performance in space.* The Journal of Strength & Conditioning Research, 2015. **29**(12): p. 3531-3545.
246. Miller, A.E.J., et al., *Gender differences in strength and muscle fiber characteristics.* European journal of applied physiology and occupational physiology, 1993. **66**: p. 254-262.
247. Courtright, S.H., et al., *A meta-analysis of sex differences in physical ability: revised estimates and strategies for reducing differences in selection contexts.* Journal of Applied Psychology, 2013. **98**(4): p. 623.
248. Watenpaugh, D.E., et al., *Lower body negative pressure exercise plus brief postexercise lower body negative pressure improve post-bed rest orthostatic tolerance.* Journal of Applied Physiology, 2007. **103**(6): p. 1964-1972.
249. Arzeno, N.M., et al., *Spaceflight-induced cardiovascular changes and recovery during NASA's Functional Task Test.* Acta Astronautica, 2013. **92**(1): p. 10-14.
250. Bishop, P., et al., *Carbon dioxide accumulation, walking performance, and metabolic cost in the NASA launch and entry suit.* Aviation, space, and environmental medicine, 1999. **70**(7): p. 656-665.
251. Hayes, J., et al., *Exercise: developing countermeasure systems for optimizing astronaut performance in space.* Biomedical results of the space shuttle program, 2013: p. 289-313.

Appendix

Table 1. Comparison of EMG Signals from BDC-5 and BDC-1 using a Student's t-test with Bonferroni adjustment.

Participant	P-value Mean-EMG(µV)	Bonferroni Corrected P-value Mean-EMG(µV)	P-value Mean-EMG_imp(µV.s)	Bonferroni Corrected P-value Mean-EMG_imp(µV.s)
PT04	0.088771243	1.775424855	0.089881135	1.79762271
PT21	0.218908185	4.378163708	0.247266949	4.94533898
PT22	0.080242294	1.604845875	0.076143554	1.52287107
PT26	0.076794828	1.535896555	0.076087611	1.52175222
PT36	0.084325146	1.686502915	0.074989492	1.49978984
PT38	0.536390672	10.72781343	0.552259152	11.045183
PT39	0.293018676	5.860373523	0.218620389	4.37240778
PT43	0.197930736	3.958614728	0.193689276	3.87378552
PT49	0.057187016	1.143740318	0.056933591	1.13867182
PT50	0.084694026	1.693880518	0.083199031	1.66398062
PT52	0.201776834	4.035536676	0.178881574	3.57763148
PT54	0.650875784	13.01751568	0.781975667	15.6395133
PT56	0.214791524	4.295830473	0.228820717	4.57641433
PT62	0.98745434	19.7490868	0.220655625	4.4131125
PT64	0.449081159	8.981623178	0.735558472	14.7111694
PT65	0.866736005	17.3347201	0.931115031	18.6223006
PT68	0.098377793	1.967555853	0.108068234	2.16136469
PT69	0.169771461	3.395429226	0.204823554	4.09647108
PT73	0.09545135	1.909026998	0.095450024	1.90900049
PT75	0.581572157	11.63144313	0.371338965	7.4267793
Mean	0.301707561	6.034151228	0.276287902	5.52575804