The impact of 14-day head-down tilt bedrest, with or without exercise, on body composition, resting energy expenditure and nutrient adequacy among healthy older adults.

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Abstract

Background. Mechanical unloading during spaceflight and prolonged bedrest causes the loss of muscle mass and strength, often leading to deconditioning of astronauts and older adults. Older adults may exhibit anabolic resistance to exercise countermeasures, exacerbated by bedrest-related challenges in achieving adequate energy intake. The planning of longer duration spaceflight requires the evaluation of alternative modes of exercise with minimal impact on energy balance. The 6-degree head-down tilt bedrest model (HDBR) is used to simulate microgravity in space and to assess physiological adaptations to mechanical unloading.

Objectives. This study assessed the effect of HDBR, with or without daily exercise, on differential changes in 1) body weight, total fat and lean soft mass measured with dual-energy X-ray absorptiometry, 2) resting energy expenditure measured with indirect calorimetry, and 3) nutrient intake analyzed by an artificial intelligence-enhanced, image-assisted food diary, in older adults.

Methods. This study was embedded in a larger two-parallel-arm (1:1) randomized-controlled trial under the umbrella of Microgravity Research Analogue (MRA). Healthy older adults aged 55-65 years were randomized to an exercise (1 hour/day of combined aerobic, high-intensity and resistance exercise) or control group (passive physiotherapy) for 14 days in a continuous bedrest position with 5-day pre- and 7-day post-bedrest monitoring. Both groups were provided a balanced weight-maintenance diet throughout. Within-group changes (pre- to post-bedrest) were determined using paired t-tests and between-group differences were determined using independent t-tests. Repeated-measures ANOVA assessed changes in resting energy expenditure, dietary intake and adequacy. Pearson's correlation tests were conducted to determine associations.

Results. Twenty-three participants were randomized and 96% completed the study (n=22, 50% women) with similar baseline characteristics (mean ± SD; 59 ± 3 y; 24.9 ± 3 kg/m^2). Significant weight loss occurred during bedrest in both exercise (Δ [95%CI]; -1.12 kg [-1.80, -0.44]) and control (-1.11 kg [-1.59, -0.64]) groups, with no group differences (*P* = 0.99). The loss of lean soft tissue mass was significant only in the control (total: -0.85 kg [-1.36, -0.33], p=0.004; leg: -0.36 kg [-0.57, -0.15], p=0.004), whereas the loss of fat mass was greater in the exercise group than in controls (-0.65 kg [-0.92, -0.38] vs. -0.26 kg [-0.46, -0.05], p=0.019). Resting energy expenditure did not change pre- and post-bedrest. Dietary protein ([% of total energy intake] 17% ± 1, 1.3 ± 0.1 g/kg/d), carbohydrate (51% ± 3) and fat (35% ± 3) intakes were comparable between groups and remained stable throughout the study. Both groups maintained adequate intakes of most nutrients throughout bedrest.

Conclusion. Healthy older adults experienced significant losses in total and leg lean soft tissue mass from 14-d of bedrest, which was partially protected by a mixed exercise protocol. Modest weight loss occurred in both groups despite efforts to increase energy intake, yet most participants achieved an overall adequate nutrient intake with a high-quality and controlled diet. Future bedrest trials should explore combinations of high-energy nutritional supplements and exercise countermeasures to further mitigate the functional declines associated with long-duration bedrest and spaceflight. Devices to provide instant assessment of body composition and components of total energy expenditure would also benefit rapid intervention during hospitalization and spaceflight.

Résumé

Contexte. La décharge mécanique pendant les vols spatiaux et l'alitement prolongé entraînent une perte de masse et de force musculaires, ce qui conduit souvent au déconditionnement des astronautes et des personnes âgées. Les adultes plus âgés peuvent présenter une résistance anabolique aux contre-mesures d'exercice, exacerbée par les difficultés liées à l'alitement pour obtenir un apport énergétique adéquat. La planification de vols spatiaux de plus longue durée nécessite l'évaluation de modes d'exercice alternatifs ayant un impact minimal sur l'équilibre énergétique. Le modèle d'alitement anti-orthostatique (position allongée avec inclinaison à moins 6-degrés tête vers le bas) simule l'environnement spatial microgravitationnel pour évaluer les adaptations physiologiques à la décharge mécanique.

Objectifs. Cette étude a évalué l'effet de l'alitement anti-orthostatique, avec ou sans exercice quotidien, sur les changements différentiels 1) du poids corporel, de la masse grasse totale et de la masse maigre molle mesurés par absorptiométrie à rayons X à double énergie, 2) de la dépense énergétique au repos mesurée par calorimétrie indirecte et 3) de l'apport en nutriments analysé par un journal alimentaire assisté par image et assisté par intelligence artificielle, chez les adultes âgés.

Méthodes. Cette étude faisait partie d'un essai contrôlé randomisé plus vaste à deux bras parallèles (1:1) sous l'égide de Microgravity Research Analogue (MRA). Des adultes âgés en bonne santé, âgés de 55 à 65 ans, ont été répartis aléatoirement dans un groupe d'exercice (1 heure par jour d'exercices combinés d'aérobie, de haute intensité et de résistance) ou dans un groupe témoin (physiothérapie passive) pendant 14 jours en position d'alitement anti-orthostatique continu, avec un suivi de 5 jours avant et 7 jours après l'alitement. Les deux groupes ont suivi un régime équilibré de maintien du poids pendant toute la durée de l'étude. Les changements au sein des groupes (avant et après l'alitement) ont été déterminés à l'aide de tests t jumelés et les différences entre les groupes ont été déterminées à l'aide de tests t indépendants. Une analyse de variance à mesures répétées a permis d'évaluer les changements dans la dépense énergétique au repos, l'apport alimentaire et l'adéquation. Des tests de corrélation de Pearson ont été effectuées pour déterminer les associations.

Résultats. Vingt-trois participants ont été randomisés et 96% ont terminé l'étude (n=22, 50% de femmes) avec des caractéristiques de base similaires (moyenne ± SD ; 59 ± 3 ans ; 24,9 ± 3 kg/m2). Une perte de poids significative s'est produite pendant l'alitement dans les groupes d'exercice (Δkg [95%CI] ; -1,12 [-1,80, -0,44]) et de contrôle (-1,11 [-1,59, -0,64]), sans différence entre les groupes (P = 0,99). La perte de masse maigre mous n'était significative que dans le groupe témoin (total : -0,85 [-1,36, -0,33], p=0,004 ; jambe : -0,36 [-0,57, -0,15], p=0,004), tandis que la perte de masse grasse était plus importante dans le groupe d'exercice que dans le groupe témoin (-0,65 [-0,92, -0,38] contre -0,26 [-0,46, -0,05], p=0,019). La dépense énergétique au repos n'a pas changé avant et après l'alitement. Les apports en protéines alimentaires ([% de l'apport énergétique total] 17 ± 1%, 1,3 ± 0,1 g/kg/j), en glucides (51% ± 3) et en lipides (35% ± 3) étaient comparables entre les groupes et sont restés stables tout au long de l'étude. Les deux groupes ont maintenu des apports adéquats de la plupart des nutriments tout au long de l'alitement.

Conclusion. Des adultes âgés en bonne santé ont subi des pertes significatives de la masse totale et de la masse des tissus mous maigres des jambes après 14 jours d'alitement, qui ont été partiellement protégées par un protocole d'exercice mixte. Une perte de poids modeste

s'est produite dans les deux groupes malgré les efforts pour augmenter l'apport énergétique, mais la plupart des participants ont atteint un apport nutritif adéquat grâce à un régime alimentaire de haute qualité et contrôlé. Les futurs essais sur l'alitement devraient explorer des combinaisons de suppléments nutritionnels à haute teneur énergétique et de contre-mesures d'exercice pour atténuer davantage les déclins fonctionnels associés à l'alitement de longue durée et aux vols spatiaux.

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Contribution of authors

As primary author I wrote the thesis, including all figures and tables. My responsibilities in this study included: screening and collecting of baseline dietary records; menu planning and preparation for all participants; recruitment and training of nutrition staff; dietary intake measurement and assessment; resting energy expenditure assessments; daily body weight measurements; verification of DXA imaging reports for body composition. I was responsible for all the statistical analysis of the data presented within the scope of the thesis.

Dr. Sonjak, Guy Hajj-Boutros, and Dr. Morais co-designed the larger randomized-controlled trial (RCT), conducted the recruitment and screening of the patients, performed body composition assessments, obtained funding, and provided expertise and guidance throughout. Dr. Morais also provided medical expertise and edited the thesis. Guy Hajj-Boutros designed the exercise intervention. Dr. Chevalier reviewed the entire thesis, provided expertise, and assisted in designing the menu, and dietary control for the larger RCT. Dr. Chevalier also assisted in designing the sub-study within the scope of the thesis.

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List of Abbreviations

AF	Activity Factor
AI	Adequate Intake
ANOVA	Analysis of Variance
BMC	Bone Mineral Content
BMI	Body Mass Index
CIM	Centre for Innovative Medicine
CO^2	Carbon Dioxide
CT	Computerized Tomography
CV	Coefficient of Variation
DXA	Dual-Energy X-ray Absorptiometry
EPOC	Excess Post-Exercise Oxygen
FFM	Fat-Free Mass
FM	Fat Mass
HDBR	6-Degree Head-down Tilt Bedrest Model
HIIT	High-Intensity Interval Training
IAAO	Indicator Amino Acid Oxidation
ISS	International Space Station
LSM	Lean Soft Tissue Mass
MPB	Muscle Protein Breakdown
MPS	Muscle Protein Synthesis
MRA	Microgravity Research Analogue
MRI	Magnetic Resonance Imaging
mTOR	Mammalian Target of Rapamycin
MUHC	McGill University Health Centre
n-3	Omega-3 Fatty Acids
O^2	Oxygen
RCT	Randomized Controlled Trial
RDA	Recommended Daily Allowance
REE	Resting Energy Expenditure
SD	Standard Deviation
TBW	Total Body Weight
TEE	Total Energy Expenditure

Introduction

A major threat to the quality of life, autonomy, and functional capacity of older adults is the progressive loss of muscle mass and quality that occurs with age, known as sarcopenia [1-4]. Sarcopenia can be accelerated by muscle disuse or bedrest, which are well-known contributors to the deconditioning of older hospitalized patients [4, 5]. Disuse-induced muscle atrophy became an important area of research after the Skylab mission of mid-1970s, as the longer spaceflight durations revealed the impact of microgravity on the loss of lean mass, and was decidedly a hazard to the functional health of astronauts and to the long-term success of missions [6]. Like microgravity, bedrest unloads tension on weight-bearing systems, causing muscle atrophy and the loss of bone mineral density [7, 8].

To alleviate the threat of spaceflight on astronauts, exercise countermeasures incorporating resistance and aerobic training are performed regularly. However, these programs often exceed two hours and are difficult to implement among the multitude of mission components that are required of the crew. Also, the efficacy and acceptability of such regimens are not well studied among older adults, despite the increasing age of astronauts. There is convincing evidence that high-intensity interval training (HIIT) is the most efficient of exercise methods for spaceflight to prevent the loss of muscle mass and bone density [9]. However, the energetic contribution of higher intensity exercise may affect important components of energy expenditure and therefore, energy requirements [10]. Changes in body composition, specifically metabolically active tissue, may also impact resting energy expenditure. A multi-modal exercise countermeasure incorporating HIIT may provide a time-efficient alternative to prevent the loss of lean mass while a shorter time commitment may help reduce the barrier to exercise.

To explore the physiological effects of microgravity, and its countermeasures, on the human body, the six-degree head-down tilt bedrest (HDBR) model has been validated as a ground-based analog to study the cardiovascular, bone, and muscle adaptations that occur in space [11, 12]. Bedrest models also enable the study of physical inactivity and benefits populations on Earth, such as hospitalized older adults. However, such studies have generally focused on younger individuals, and little is known about the efficacy of exercise countermeasures incorporating HIIT on mitigating muscle atrophy in bedridden older adults. Due to the ageing population, the increasing average age of astronauts, and the advent of commercial spaceflight [13], it is increasingly important to prioritize research identifying countermeasures for disuse-related disorders to prevent the decline of astronauts' functional capacity, and to promote healthy aging.

This research was designed to characterize the effect of a 14-day head-down tilt bedrest among older adults (55-65 years) undergoing either one hour/day of multi-modal exercise or passive physiotherapy on changes in body composition, resting energy expenditure (REE), and nutrient adequacy. The study also explored relationships between body composition, REE, and dietary intake, as well as limb muscle changes in response to nutrient adequacy. The sub-study was embedded within a larger single-center bedrest randomized-controlled trial (RCT) under the umbrella: Microgravity Research Analogue (MRA): Understanding the health impact of inactivity for the benefit of older adults and astronauts Initiative.

Chapter 1: Literature Review

1.1 Mechanical unloading and functional decline during spaceflight and ageing

Skeletal muscle is highly responsive and adaptive to changes in its mechanical environment. Mechanical unloading refers to a diminished resistance on the musculoskeletal system which leads to the loss of lean mass and functional capacity [14]. Among the various settings in which this occurs -such as immobilization, bedrest, physical inactivity, and spaceflight- microgravity in space has the most pronounced effect on skeletal muscle tissue [11]. Two weeks of spaceflight has shown to produce a 20% decrease in mean skeletal muscle mass, and up to 50% decrease in mean muscle strength [14]. The muscles most affected are involved in maintenance of an upright posture in gravitational environments, such as the leg and trunk musculature. Although the duration of mechanical unloading is an important predictor of the severity of muscle atrophy, substantial losses can appear within 2-3 days [15].

Human safety and health are a primary concern when considering further space exploration [7]. Therefore, the planning of longer-duration spaceflights (≥1 year), such as for Martian exploration, will require improved countermeasures to prevent functional losses during prolonged microgravity exposure. Previous efforts to determine safety protocols for spaceflight have focused on younger individuals, explaining the dearth of evidence to protect older adults from the conditions in space, such as radiation exposure, isolation, microgravity, and depressed immune function [16]. Given the evidence of physiological differences between older and younger individuals in their response to spaceflight conditions, there is a necessity to examine the efficacy of countermeasures, such as exercise, among healthy older populations.

An important overlap exists between adaptations to microgravity and ageing on earth. Sarcopenia is defined as an age-related process that is characterized by the progressive loss of muscle mass and strength, contributing to adverse outcomes such as increased occurrence of falls and hospital readmissions, reduced autonomy, and mortality [2-4]. The prevalence of sarcopenia is high in hospitalized older adults, approximately 23% [95% CI: 15-30%) in men and 24% [14-35%] in women [17-19]. As such, the preservation of skeletal muscle mass and function should be a key focus in research and clinical settings to extend the number of years that an individual spends unburdened by health complications. Bedrest is considered to be a key driver in the etiology of age-related sarcopenia, and is commonly indicated in hospitals due to surgery or acute illnesses [20]. While healthy ageing may be associated with decreases in muscle mass of ~0.5-1% per year after the age of 50, a comparable rate of loss can be experienced within just two to three days of bedrest [20]. Older adults undergoing bedrest may exceed the rate of muscle loss of their younger counterparts by as much as 3-6 times [2]. Importantly, the loss of muscle strength is strongly correlated to - and often exceeds that of- muscle mass when controlled for age, sex, bedrest modality and duration [adjusted $r^2 = 0.790$, $P = 0.001$] [20]. Also, older individuals are generally less active and have a lower baseline fitness, which reduces the likelihood that losses are recoverable. Zisberg et al., reported that half of older patients who were admitted for a non-disabling condition suffered from long-term (≥1 month) functional decline following discharge, which was attributed to the state of low mobility among hospitalized patients [5]. As such, countermeasures to mitigate the effects of bedrest are should be optimized to prevent functional declines and to extend an individual's quality of life.

1.2 Head-down tilt bedrest: a ground-based analog of microgravity

Among other conditions in space -such as radiation exposure, distance from earth, and isolation- microgravity has the broadest effect across systems of the human body, with the musculoskeletal system being the most affected [7, 14]. Ground-based analogs of microgravity have been validated to simulate the its effect on the human body in order to bypass the logistical and ethical challenges associated with the cost and hazards of conducting studies in space [7, 21]. Such analogs include dry and wet immersion, single-limb immobilization, supine bedrest, and head-down tilt bedrest (HDBR). Although each model presents different strengths and limitations regarding their application to actual microgravity, HDBR is uniquely able to reproduce the head-ward fluid shift present during weightlessness and is the international standard for studying musculoskeletal and cardiovascular adaptations to microgravity [22]. Bedrest studies also have the benefit of being applicable to hospitalization, which has contributed to our understanding of the deleterious effects of inactivity in young and older adults [23].

Bedrest studies are not without their limitations, as they require prolonged hospital stay, extensive screening protocols and post-bedrest monitoring, often resulting in smaller sample sizes to manage the costs and logistics of conducting such a demanding study [24]. There are also important differences between the effects of HDBR and microgravity on the musculoskeletal system. While astronauts experience a near-complete unloading from gravitational forces, HDBR only alters the gravity vector across the entire body from anterior to posterior [24]. The greater degree of mechanical unloading present in microgravity assumes a more rapid onset and severity of muscle atrophy than ground-based models [7]. On earth, HDBR studies have shown to increase diuresis, which also differs from spaceflight where total body water largely remains unchanged [16]. An important discrepancy exists between the typical participants of bedrest studies and astronauts. While the median age of bedrest participants has been reported as 24.5 y, the average age of astronauts has increased from 40.9 to 45.3 y from the first to last recorded spaceflights, respectively [25-27]. Only a small number of bedrest studies have involved older adults [28]. This disconnect emphasizes the need to address the knowledge gap on the effects of bedrest and related countermeasures on older populations.

1.3 Mechanisms of changes in body composition during bedrest

The loss of skeletal muscle mass must be underpinned by an imbalance in muscle protein turnover which is determined by rates of muscle protein synthesis (MPS) and muscle protein breakdown (MPB). The absence of muscle tension is a potent inhibitor of MPS through the mammalian target of rapamycin (mTOR) anabolic signaling pathway [29, 30], and studies in microgravity and bedrest have consistently shown a decrease MPS rates [31]. Kilroe et al [32] demonstrated this by applying oral deuterated water dosing methods to determine the impact of disuse on free living and daily myofibrillar protein synthesis rates during 1 week of single-limb immobilization in healthy young men (n = 13, age = 20 ± 1 y, BMI = 23.4 ± 0.9 kg/m^2). Muscle biopsies were collected before and after the period of disuse and revealed a 36 ± 4% lower myofibrillar protein synthesis rate in the immobilized leg compared with the non-immobilized control leg ($P < 0.001$). While studies in microgravity and bedrest have revealed lower MPS rates in young and older adults [2, 31, 33, 34], it is increasingly clear that MPB rates either decrease or remain unchanged [35]. Consequently, it has been suggested that disuse-related muscle atrophy is driven primarily by impairments in MPS [36]. Decreases in the rate of MPS are more pronounced during the first 3-14 days of unloading and eventually reaches a plateau where muscle atrophy occurs at a slower rate [20, 31]. The differential changes in MPB and MPS rates may account for the non-linear trajectory of muscle loss observed during disuse [20]. Mechanical unloading leading to declines in lean mass are well documented [37], in addition to an increasing fat infiltration into muscle [38, 39]- which is postulated to be a central aspect of sarcopenia and indicative of future mobility limitations [40]. During bedrest and physical inactivity, the onset of insulin resistance and decline in basal metabolic rate may also contribute to the overall accumulation of adipose tissue. Bedrest has shown to significantly worsen hepatic insulin sensitivity and leads to dysregulations in glucose and fat metabolism in older, overweight individuals [41]. A decrease in lipolysis has been shown to precede the onset of insulin resistance, however the direct mechanism remains inconclusive and complex [41].

1.4 Measuring body composition changes during bedrest

The measurement of body composition can be accomplished by various methodologies that differ in how they compartmentalize and assess tissues in the human body. To answer the question of how body composition changes during bedrest, the methodology selected should be able to differentiate between components of lean tissue (i.e., bone and soft tissue) and fat tissue. Although computerized tomography (CT) imaging is regarded as the gold-standard imaging method for body composition analysis, it is not safe for whole-body assessment and for repeated measurements required to assess changes in body composition due to the high radiation dose [42, 43]. Magnetic resonance imaging (MRI) has an advantage over CT imaging as it does not use ionizing radiation for imaging. Instead, a magnetic field is generated to determine body composition using tissue-specific magnetic resonance properties with excellent accuracy [44]. However, the limitations of whole-body MRI and CT assessments are that they are costly, and their access is limited for research purposes. As such, dual-energy X-ray absorptiometry (DXA) is the most practical and commonly used imaging tool for whole-body assessments in research settings [42].

Dual-energy X-ray absorptiometry is a noninvasive technique that uses two low-radiation X-ray beams to measure body composition through tissue-specific attenuation properties. It provides quantification of the major body compartments including bone mineral and soft tissue, with the latter divided into fat and fat-free tissue. Although the terms *lean body mass* and *fat-free mass* (FFM) are often used interchangeably, they represent different body compartments. *Lean body mass*, more correctly termed *lean soft tissue mass* (LSM), is the sum of total body water, protein, carbohydrates, non-fat lipids, and soft tissue mineral. While changes in FFM and LSM are often correlated due to the critical contribution of skeletal muscle mass to both compartments, it is important to differentiate these when assessing body composition in specific studies as FFM includes the sum of bone mineral content (BMC) and LSM [42]. DXA allows for this differentiation through a 3-compartment model, and is the reference method for estimating whole-body lean soft tissue mass and fat mass with a high test-retest precision of 2% (CV) [45]

1.5 Therapeutic countermeasures and nutritional factors to address muscle atrophy associated with mechanical unloading

Muscle atrophy during bedrest and spaceflight is virtually inevitable without the use of exercise or nutritional countermeasures. There is currently no standalone countermeasure to prevent the detrimental effects of microgravity or prolonged bedrest [46]. Bedrest studies and in-flight observations consistently indicate that an integrated exercise protocol should be combined with adequate intake of key nutrients and energy to mitigate muscle atrophy [6, 37, 46, 47]. Since it has been concluded that human skeletal muscle atrophy is predominantly driven by the downregulation of MPS, identified countermeasures are those involved in the activation of the IGF-1-Akt-mTOR pathway, a key regulator of the translation step in MPS [30, 37]. This signaling pathway is known to be sensitive to stimuli such as certain nutrients, growth factors, energy balance and mechanical tension or muscle contraction [6, 48, 49]. Older adults may exhibit anabolic resistance or an impaired efficiency of utilizing such stimuli to regulate protein synthesis, which contributes to higher protein and leucine needs compared to younger adults to attenuate the loss of muscle mass in older adults [33, 50, 51]. Therefore, responses to bedrest and exercise countermeasures must be evaluated separately for older adults to design suitable and effective protocols to mitigate muscle atrophy during long-duration spaceflight and bedrest.

1.5.1 Exercise countermeasures for muscle atrophy during spaceflight and bedrest

Performing regular exercise in space and on Earth is regarded as the most effective strategy to preserve muscle mass, attenuate functional losses, and to support the re-adaptation to muscle re-loading [7, 8]. Exercise countermeasures are likely to be required for periods of unloading greater than 14 days to prevent a significant deterioration in functional capacity [47]. Current obligatory aerobic and resistance training protocols by international space agencies are time-intensive, requiring 2.5 hours of exercise per day, 6-7 days per week [52]. This could result in a negative energy balance if food intake fails to supply the increased energy needs due to physical activity energy expenditure [53, 54]. A systematic review and meta-analysis by Comfort et al. [21] also found that low-moderate intensity resistive exercises are insufficient for the maintenance of muscle mass during prolonged spaceflight. In the context of planning longer duration missions for planetary exploration, it is imperative to evaluate alternative modes of exercise with minimal impact on components of energy balance (i.e., expenditure, intake) [6, 15, 46]. High-intensity interval training (HIIT) may fulfill these needs by diminishing the amount of time spent exercising while preventing the loss of lean mass [53].

Moro et al [55] compared the effect of 2-mo of high-intensity interval resistance training (HIIRT, 45 minutes) versus traditional resistance training (TRT, 65 minutes) in healthy older adults (n= 35, age= 60-80 y, BMI= 22.1-29.5 kg/m^2) and demonstrated a higher retention rate in the HIIRT group (79% vs 53%). Both exercise methods showed comparable increases in strength, measured by 3-6 rep max strength tests, while the shorter time commitment required of the HIIRT group may have helped to reduce the barrier to exercise. Some studies among ambulatory adults have associated a greater metabolic cost with HIIT due to the heightened post-exercise oxygen consumption and basal metabolic rate in ambulatory subjects [56]. Also, long durations of high-intensity exercise may not be well tolerated or accepted among older adults. To achieve a balance between the time spent exercising and relative acceptability of the exercise countermeasure, a *multi-moda*l training program may provide an efficient and effective alternative to *unimoda*l exercise countermeasures. A meta-analysis by Wilson et al. investigated the effects of combining resistance training with HIIT and found that participants who performed concurrent training experienced greater increases in muscle mass and improvements in cardiovascular fitness when compared to those who only performed resistance training [57].

While resistance training has well-known effect on muscle hypertrophy, aerobic exercise has generally been thought to interfere with this process. The latter has been attributed to the activation of catabolic pathways and release of cortisol, which can promote muscle breakdown and inhibit muscle growth [58]. In addition, aerobic exercise can deplete glycogen stores, which are necessary for muscle growth, without adequate replenishment [59]. However, recent literature has suggested that aerobic exercise can promote muscle hypertrophy when combined with resistance training by increasing blood flow to muscles and promoting protein synthesis in the mitochondrial subfraction [57]. Aerobic training also improves cardiovascular fitness, which can enhance resistance training performance and lead to greater gains in muscle mass [60]. These findings highlight the importance of proper exercise programming for mitigating muscle loss in a context such as bed rest. There are currently no studies which have evaluated the efficacy of a multi-modal training program (including components of HIIT, resistance, and aerobic exercise) in mitigating muscle atrophy, nor its impact on resting energy expenditure, among bedridden and healthy older adults.

1.5.2 Energy balance impacts the maintenance of lean mass during spaceflight and bedrest

Since the first moon landing in 1969, nutritional research has played a critical role in maintaining the health of astronauts, from providing sufficient energy to meet the metabolic cost of daily exercise protocols, to the maintenance of immune, endocrine, and musculoskeletal systems [61]. Exercise and adequacy of energy and key nutrients (e.g., protein and leucine) act synergistically to promote MPS and mitigate muscle atrophy during periods of inactivity. Weight loss and energy intake in space are highly variable, but astronauts often do not meet their energy requirements for a variety of reasons. Consuming enough calories to meet the metabolic demands of obligatory exercise can be challenging for astronauts, despite the sufficient availability of food onboard [62]. Matsumoto et al. reported an estimated rate of body weight loss of 2.4% per 100 days via linear extrapolation from previous space missions (n = 619 missions), while Smith et al. evaluated the available data on energy intake in space flight (since the 1980s) and reported an average consumption of 70% of estimated energy requirements [63, 64]. Similarly, malnutrition is prevalent in hospitals due to illness effects, eating difficulties, and organizational factors [65]. While increased energy expenditure associated with the catabolic effect of certain illnesses can be confounding to the causes of energy imbalance, bedrest alone may contribute to decreases in appetite and energy deficits [66, 67]. Negative energy balance, either by inadequate intake or greater expenditure, compromises the maintenance of amino acids reserves within the muscles to provide precursors for gluconeogenesis [68]. Evaluating the impact of exercise countermeasures on energy expenditure is primordial to understanding energy requirements for the maintenance of astronaut's health and performance as mission duration increases in length [68].

1.6 Measuring the impact of exercise on resting energy expenditure during bedrest

Gaesser and Brookes (1984) first identified an elevated oxygen uptake during the recovery period post-exercise which was termed "excess post-exercise oxygen consumption" (EPOC) [69]. The intensity of exercise has been positively associated with greater changes in EPOC, which may in turn increase the energy cost of exercise through changes in basal metabolic rates. Resting energy expenditure (REE) is defined as the energy required to maintain the body's basic metabolic activity and vital functions (ie., respiration, body temperature) in a fasted and rested state. It is a primary component (60-75%) of total energy expenditure (TEE), followed by activity energy expenditure and the thermic effect of food [70]. REE is largely determined by metabolically active tissue- mainly lean soft tissue mass which constitutes skeletal muscle, organs, and water- with organs being the most important contributor (75%), followed by skeletal muscle (20%) [71]. In the context of bedrest, decreases in skeletal muscle mass may reduce REE and impact energy balance and consequently, the anabolic response to exercise. Notably, the complex relationship between bedrest, high-intensity exercise, and REE have not yet been explored in older populations.

Direct calorimetry is the gold standard for REE measurement for its high accuracy and precision (error range of <1%), it is more invasive and expensive when compared to indirect calorimetry- it is also unfeasible given in the context of bedrest studies where participants are sharing bedrooms. Indirect calorimetry is a practical and reliable alternative for measuring REE and relies on the assumptions that glucose, fatty acids, amino acids, and oxygen (O^2) are the substrates for energy production in humans which results in the by-products heat, water, and carbon dioxide (CO^2) [72]. The substrates are also oxidized according to a specific fixed respiratory quotient (RQ) calculated as CO^2 released/O^2 absorbed. The final assumption is that the loss of substrates are negligible in urine and feces. The abbreviated Weir equation (REE (kcal/d) = [(3.94 x VO^2 (L/min)) + (1.1 x VCO^2 (L/min)] x 1440) accounts for the nitrogen lost and only requires the measurement of gas volumes to calculate REE. Although there are some limitations with the underlying assumptions of the method, indirect calorimetry is able to reliably measure REE with an error range of 5-10% and provides measurements that are in close agreement with direct calorimetry [73].

1.7 Nutrient adequacy may affect responses to exercise during bedrest and spaceflight

Since previous bedrest studies and observations during spaceflight have shown a reduction in energy intake, a very high-quality diet may be required to provide adequate nutrients below a certain energy intake threshold. Nutrient intake may also be compromised in response to potential decreases in resting energy expenditure (REE) and subsequent reduction in food intake [74, 75]. In the presence of negative energy balance, it is generally accepted that higher protein intake (1.2 g/kg body weight/d) may maximize the MPS response to feeding and to assist in the maintenance of muscle mass [48]. Ageing and muscle disuse are also associated with anabolic resistance to protein feeding [76], which are partly responsible for the progressive loss of skeletal muscle mass observed in older adults [77]. Since performing exercise and increasing energy or protein intake may not always be feasible in hospitalized patients, several nutrients have been studied to protect against losses in lean body mass. Among the most widely studied nutrients for this purpose is leucine, an amino acid that is considered a strong stimulator of MPS [78]. Szwiega et al. [51] evaluated leucine requirements of a small number of healthy elderly individuals using the indicator amino acid oxidation (IAAO) method, and found that requirements were nearly double of young adults based on the international guidelines published by the World Health Organization (78.2 mg/kg/d vs 39 mg/kg/d). However, English et al. [79] found that leucine supplementation (0.06 g/kg/meal) did not mitigate the loss of whole-body lean mass during 14 days of bedrest among older adults, despite leucine intake being comparable to the requirements determined using the IAAO method. Overall, studies evaluating the efficacy of leucine supplementation on muscle maintenance have revealed inconsistent results, and may not translate to a real improvement in functional capacity [80].

Other strategies to prevent and treat age-related muscle atrophy have focused on overcoming anabolic resistance, which has led to novel discoveries suggesting the importance of non-protein nutrients for the regulation of protein turnover [81]. Omega-3 (n-3) fatty acids may increase the sensitivity of anabolic pathways to stimuli through alterations in skeletal muscle phospholipid membranes' content of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) [82]. McGlory et al. [83] have previously demonstrated the efficacy of a daily dose of omega-3 fatty acid (n-3; n = 11; 5 g EPA + DHA) when compared to controls (n = 9; isoenergetic and volume equivalent sunflower oil) in attenuating muscle atrophy (14 vs 8%, $P < 0.05$) during two weeks of unilateral leg immobilization in healthy young women (age = 22 ± 3 y, BMI = 23.0 ± 2.3 kg/m^2). The study also observed higher integrated rates myofibrillar protein synthesis in the n-3 supplementation group when compared to controls and was suggested to be a mediating factor in the attenuation. Vitamin D is another nutrient of interest as it's deficiency has shown to lead to oxidative stress and decreased mitochondrial function in skeletal muscle, which has been suggested to contribute to muscle atrophy [84]. However, a systematic review and meta-analysis by Antoniak et al. [85] evaluated the additive effect of vitamin D_3 on resistance exercise and found no additional benefit of supplementation on functional outcomes (short physical performance battery, timed up and go). However, the study only evaluated in seven studies (n = 792) and included one high-risk study and could not provide a firm conclusion on the efficacy of Vitamin D supplementation on muscle outcomes. Based on the evidence presented, intakes of leucine, n-3, and Vitamin D may contribute to the maintenance of muscle mass in the context of bedrest and spaceflight.

1.7 Rationale

Mechanical unloading of the body in microgravity and bedrest causes the loss of lean mass and body weight, and changes in body composition- which are hazardous to the health of astronauts and bedridden patients. The six-degree head-down tilt bedrest model (HDBR) allows for a controlled evaluation of the effect of mechanical unloading on human health. Such studies have largely included younger adults, due to the greater health risks associated with prolonged bedrest among older populations. However, the average age of astronauts is increasing and warrants greater assessment of the effectiveness of exercise countermeasures on mitigating unloading-induced losses in lean mass in older adults. The effect of a daily multi-modal exercise countermeasure on healthy older adults undergoing prolonged bedrest has not yet been investigated. Changes in body composition may also play an effect on resting energy expenditure (REE)- a major contributor to total energy expenditure and therefore, energy balance. The impact of exercise countermeasures on REE within bedrest conditions has also not been investigated in this population.

1.8 Objectives

1. To characterize and compare the effect of HDBR, with or without 1 hour/day of multi-modal exercise, on differential changes in body composition, REE, and nutrient adequacy among healthy older adults (55-65 years old).
2. To assess within-group relationships between changes in lean mass, components of energy balance (i.e., nutrient intake and REE), and adequacy of key nutrients involved in muscle protein synthesis among bedridden older adults.

3. To explore potential challenges of dietary intake and energy balance during bedrest, and to explore their significance to spaceflight and hospitalization.

1.9 Hypotheses

It was hypothesized that 14 days of bedrest would result in a greater decline in physical activity and loss of total and leg lean soft tissue mass in the non-exercising group. As compensatory responses to the decline in lean mass, proportional reductions in REE and energy intake would occur, such that nutrient adequacy may be compromised and lead to greater within-group losses in lean mass. It was hypothesized that a multi-modal exercise program would mitigate those changes, such that the proposed countermeasure could be tested in clinical, research, and spaceflight settings to prevent muscle atrophy and the functional decline of healthy older adults.

Chapter 2: Methods

2.1 Study Population

Details on the study design, recruitment, methods and primary results will be published in a forthcoming methodology paper for the larger randomized-controlled trial. Briefly, twenty-nine participants aged 55 to 65 years were recruited from the greater Montreal and Toronto regions through social media and print advertisement. Because of the physically and mentally demanding nature of the study, eligible participants had to be healthy and active. Potential participants were males and females (menopaused > 1 year) with a BMI between 20-30 kg/m^2 who performed ≥ 2.5 h/week of exercise of moderate to vigorous-intensity aerobic activity. The height was constrained (158 cm to 190 cm) due to the limitations of DEXA (and MRI in the larger RCT). Initial screening was conducted over the telephone and/or email, covering basic inclusion criteria, including physical activity questionnaires (Physical Activity Scale for the Elderly, Get Active Questionnaire), frailty questionnaire, and a psychological questionnaire to assess their physical and emotional fitness. Following the initial phone screening, participants were thoroughly screened, following a protocol which included a medical examination (e.g., blood and urine collection, physical function measurement, and psychological interview) before they were considered for inclusion in the study.

Exclusion criteria are detailed in **Table 1**. In summary, these included: presence of dementia and/or mental illness, history of cardiovascular and/or lung disease, central and/or peripheral nervous system disease or alterations, orthostatic intolerance (frequent fainting and light-headedness), anemia or low platelet count, risk for thrombosis, presence of severe allergies. All participants provided their written informed consent regarding the nature of the study and details of experimental protocol in accordance with the Declaration of Helsinki. The study was approved by the McGill University Health Centre (MUHC) research ethics board and registered on ClinicalTrials.Gov Identifier: NCT04964999.

Table 1: Inclusion and Exclusion Criteria

Inclusion Criteria:

Healthy male or female (55-65 y), if female: menopausal (>1 y)
Non-smoker
Height: 158-190 cm
BMI: 20-30 kg/m^2
Psychological and medical screening passed
≥2.5 h/week of exercise (moderate to vigorous-intensity aerobic activity)

Exclusion Criteria:

Presence of dementia and/or mental illness
History of cardiovascular disease and/or lung disease
Central and/or peripheral nervous system disease or alterations
Difficulties with upright posture (standing), frequent fainting and lightheadedness
History of head trauma
Have anemia or low platelet count
Are at risk and/or have family history of thrombosis
Presence of severe allergies
Kidney problems (e.g., renal stones, etc.)
Low calcium levels
Vestibular disorders (e.g., vertigo, dizziness, trouble with balance, migraines, etc.)
Presence of musculoskeletal issues (e.g., neck, back, or knee pain, dull aches, swelling, stiff joints, etc.)
Osteoporosis, seizures, presence of ulcers, acid reflux, hiatal hernia
Refusal to conform to prescribed diet, or who object to frequent blood collection
Special dietary requests (e.g., vegetarian, vegan, or some other diet)
Recent lack of proper nutrition (malnourishment)
Have HIV and/or hepatitis B and/or hepatitis C
Taking medications that may interfere with the interpretation of the study results
Claustrophobia
Metallic implants (pacemakers, ICDs, CRT devices, infusion pumps, cerebral artery aneurysm clips, dental implants, tissue expander, etc.), osteosynthesis material
Given blood in the past 3 months before the onset of the study
Smoked within 5 months prior to the start of the study
Abused drugs, medicine, or alcohol within 30 days prior to the start of the study
Drunk and/or alcohol addiction
Participated in another study within 2mo before study onset
A positive COVID-19 test taken 1 week to 24h before study start date
Not willing to share a room with another person

2.2 Overview of Study Design

This sub-study was embedded within a larger single-center bedrest randomized-controlled trial (RCT), *The Inactivity Study*, under the umbrella: *Microgravity Research Analogue (MRA): Understanding the health impact of inactivity for the benefit of older adults and astronauts Initiative.* The overall objective of this sub-study was to assess the effect of six-degree head-down tilt bedrest (HDBR), with or without a multi-modal exercise protocol, on changes in body composition, resting energy expenditure, and adequacy of nutrient intake in healthy older adults. Eligible participants who provided written consent (n = 23) underwent stratified randomization (block size, 4; strata, 2: men/women) with a treatment allocation ratio of 1:1 to either control (n = 12) or exercise (n = 11) groups prior to bedrest using an online Simple Randomization Software (Version 1, Sealed Envelope Ltd, London, UK). Blinding was not possible for the participants nor study personnel due to the nature of the treatment. Participants were admitted at the Center for Innovative Medicine (CIM) of the Research Institute of the MUHC (Montreal, Quebec, Canada) for 26 days. The study was divided into the following 3 periods: 1) a 5-day baseline or adaptation period (B), when participants were ambulatory and underwent baseline assessments, 2) a 14-day head-down tilt bedrest period (BR), where the exercise group took part in a daily multi-modal exercise program, while the control group participated in physiotherapy exercises, 3) a 7-day recovery period (REC), where all participants were ambulatory, monitored, and underwent recovery assessments prior to leaving the facility, as shown in **Figure 1.** Apart from the exercise sessions, there was no difference in treatment between the two groups.

Figure 1: Schedule of study and experimental procedures

	Baseline period (B)					**Bedrest period (BR)**														**Recovery period (REC)**						
Study Day	1	2	3	4	5	1	2	3	4	5	6	7	8	9	10	11	12	13	14	1	2	3	4	5	6	7
Dietary intake	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Weight	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
REE				X			X					X							X							
Body composition			X																			X				
Exercise session						X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Physiotherapy session						X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Visit duration	**26 days**																									

2.3 Study Protocol

2.3.1 Head-down tilt bedrest period (BR)

During this period, participants were confined to a strict 6° head-down tilt bedrest position and monitored via an alarm that set off if participants elevated their body or head. Facility personnel at the RI-MUHC ensured round-the-clock compliance. All leisure activities, eating, watching television, and all personal hygienic procedures (e.g., showering, teeth brushing, shaving) took place in a HDBR position for the duration of this period. For mealtime, the participants adopted the prone position, which consists in laying on their abdomen with the food presented to them on a lower table. They were also allowed to lie on the side for meals. Bedpans and urine bottles were used for bowel movement and urination. Because many participants experienced headache, backache, and/or constipation during BR, acetaminophen was administered by the research nurse for pain, and docusate sodium was prescribed as needed for stool softening.

2.3.2 Exercise Intervention and Control Group

The rationale for the exercise countermeasure has been described in greater detail elsewhere [27]. Briefly, during the BR period, the control group underwent daily physiotherapy stretching sessions while the intervention group followed a daily multi-modal exercise countermeasure. The intervention consisted of three exercise sessions per day, totaling one hour daily, with approximately four hours between sessions. The countermeasure protocol incorporated high-intensity interval training (HIIT), aerobic, and resistance exercises while maintaining the head-down tilt position (Table 2). HIIT and aerobic exercises were conducted using cycle ergometer (Ergoselect 8, Ergoline) and the intensity was individualized to attain a pre-specified heart rate reserve (%). Resistance training exercises were divided into upper (e.g., external shoulder rotation, chest fly, lateral pull-down, and dead bug) and lower body (e.g., hip raise, leg press, ankle pump, leg curls) exercises (3 sets, 10-12 repetitions) using cables, resistance bands, and body weight. Exercise details were recorded to determine the level of compliance to the study treatment and will be reported in a forthcoming paper.

Table 2. Exercise countermeasure protocol for the bedrest study

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

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

HRR, heart rate reserve.

Table 2. Exercise countermeasure protocol for the bedrest study in older adults, reproduced with permission from Hedge et al., [27]. The exercise group underwent two weeks of a multi-modal exercise countermeasure incorporating HIIT, aerobic, and resistance training. Three sessions per day were performed and focused on 1-2 components of the program. Exercise duration, equipment, and descriptions of each component are detailed.

2.3.4 Study Diet and Preparation

A balanced and isocaloric diet was provided by the facility to the participants during all dietary-controlled periods (ie., baseline, bedrest, and recovery). To ensure adequate energy intake during baseline, daily energy requirements were estimated using the Mifflin equation [86]. Before the bedrest period, resting energy expenditure was measured with indirect calorimetry to calculate a more precise prediction of energy requirements for the remainder of the study. For these calculations, an activity factor (AF) of 1.5 was used for ambulatory periods. During bedrest, the AF for the control group was reduced to 1.3 to account for reduced physical activity, while the exercise group maintained the ambulatory diet to ensure adequate caloric intake to support the energy requirements of exercise. Ten percent of the total energy expenditure (TEE) was added to account for thermogenesis (i.e., $(REE\ (kcal/d) \times\ AF) + (0.1\ \times\ TEE))$.

Meals, snacks and beverages were prepared to meet the calculated TEE and specific nutrient requirements as specified in Appendix A. The standardized diet consisted of 55% carbohydrate, 30% fat, and 15% protein macronutrient content of total energy. Recommended intakes of vitamins and minerals were to be achieved as an average per week. Protein and leucine requirements were prespecified as 1.2 g protein/kg body weight/day and 6-7 g/day distributed among 3-4 meals. To reach targets and avoid seasonal differences all participants were supplemented with 1000 IU vitamin D3. To minimize bone loss during bedrest, 1200 mg of calcium supplements were provided daily for all participants. Besides meeting nutrient requirements, some dietary restrictions were mandatory, such as: 1) methylxanthine derivatives limited to 2 cups per day (coffee, decaffeinated coffee, black and green tea, energy drinks, chocolate, cola), 2) no alcohol intake, 3) no flavor enhancer 4) no sweat inducing spices (such as chili, hot curry).

Main meals were prepared by Les Fermes PB (Marieville, QC, Canada), according to our specifications to contain >20 g protein per serving and >15 g protein for vegetarian options. Meals were delivered and kept frozen until use. All side dishes were prepared from fresh ingredients in a metabolic kitchen by the study nutrition staff. Meal options were provided to participants every night to predefine the next day's menu for each participant according to their preferences, nutrient requirements, and test schedule. Participants were encouraged to finish their plate and were allowed to request snacks ad libitum. Meal and snack offers were adjusted if participant's body weight deviated by ± 3% of their baseline. To determine and control for individual nutrient intake throughout 26 days (B, BR, REC), all quantities of consumed items were documented and evaluated by the trained nutrition staff. In case of any leftovers, an estimation of their nutrient content was calculated by the nutrition software and the missing part of the nutrients was provided to the participants with the next meal to supply a standardized level of daily nutrients.

2.4 Measurements

2.4.1 Anthropometrics and Body Composition

Height was measured once during screening to the nearest 0.5 cm on a standardized wall-mounted height board. Weight was measured daily to the nearest 0.1 kg using a standing scale (H350, Balance Canada) during ambulatory periods and a scale integrated in the hospital bed (Bari10A, Stryker) during the bedrest period, with participants dressed in light clothing without shoes. Body composition was measured twice (before and after BR) using DXA scanning (Lunar iDXA, General Electric Lunar Prodigy, Madison, WI, USA). Participants lay on an open scanner for approximately 10 minutes to determine whole-body and regional lean soft mass (LSM), bone mineral content (BMC) and fat mass (FM). Each individual report was verified to ensure that regions of interest coincided with the appropriate landmarks for appendicular and trunk regions.

2.4.2 Resting energy expenditure (REE)

REE was measured by continuous indirect calorimetry using a ventilated-hood metabolic monitor (TrueOne 2400, ParvoMedics, Sandy, UT) at 4 timepoints (once during B, 3 times during BR, see Table 1), at 7h00. Before each test, the calorimeter was calibrated with the use of a reference gas mixture (96% oxygen and 4% carbon dioxide) obtained from the manufacturer. All participants were fasted overnight for a minimum of 10 h prior to measurement, with only water permitted. Participants lay in supine position in a thermally neutral (20-27^{o}C), quiet room before beginning. Participants were instructed to minimize movement, and to remain as quiet as possible during the testing period. Participants breathed under the plastic canopy for a minimum of 20 minutes. Data from the first 5 minutes were excluded; the average of the last 15 minutes were used for calculation of the 24-h REE based on the Abbreviated de Weir equation [87]. Minutes that were associated with movement, if any, were excluded from the analysis. Test results with an intraindividual coefficient of variation of less than 5% were included in the analysis.

2.4.3 Nutrient adequacy

Nutrient intake was measured during baseline, bedrest, and recovery periods using an AI-based, image-assisted mobile app (Keenoa, Montreal, Quebec, Canada) which has been validated against ASA24 to assess usual intakes [88]. The app obtains dietary data from the Canadian Nutrient File v2015, the USDA National Nutrient Database, and frequently important food items from the South Korean Food Composition Database, the Hong Kong Nutrient Information Inquiry System, the Indian Food Composition Tables, and the Australian Food Composition Database. Nutrient adequacy was reported as percentages and was calculated by dividing the periodic means of daily nutrient intakes over age and sex-specific dietary reference intakes (DRI) [89]. The DRI referred to the nutrient's Recommended Daily Allowance (RDA) when available, otherwise Adequate Intake (AI) levels were used. Macronutrient intake was compared to the Acceptable Macronutrient Distribution Ranges (AMDR) and all nutrient targets are specified in **appendix A**. The cut-off point method was used to determine nutrient adequacy and was defined as the upper quartile of the DRI (≥75%) to avoid overestimating the actual level of adequacy among participants [90]. Recent work using the indirect amino acid oxidation method has revealed a greater dietary leucine requirement for older adults of 78.2 mg/kg/d due to age-related differences in leucine metabolism, therefore, this standard was used to assess leucine adequacy **[51]**.

2.5 Statistical analysis

Continuous variables were shown as means ± SD, unless stated otherwise. Data was verified for normality (Shapiro Wilk's test) and homogeneity (Levene's test for t-tests, Mauchly's test for sphericity for repeated-measures ANOVA). Greenhouse-Geisser and Huynh-Feldt corrections were applied for departures from sphericity. Pre – post-bedrest changes in body composition within groups are presented as change and 95% confidence interval of the change, compared by paired t-tests, and group differences were determined by independent t-tests. REE and nutrient adequacy were analyzed with repeated-measures ANOVA (within-group factor: period, between-group factor: group); period-by group interactions indicate differential changes over time between groups. When ANOVA revealed significant differences, Tukey's post-hoc test with Bonferroni adjustment were performed. Pearson's correlation were used to explore associations between continuous variables at single timepoints. All randomized participants who completed the BR period were included in the analysis. The significance level was set a priori at $p < 0.05$ and all analyses were conducted using R software (version 4.1.1).

Chapter 3: Results

3.1 Participants

Twenty-two randomized participants completed the study and rigorously adhered to the bedrest and exercise protocol. There was an equal sex ratio (45.5% male) in each group, the mean age (± SD) of participants was 59 ± 4 years and all participants were at a healthy BMI (24.9 ± 2.8 kg/m^2) and without co-morbidities, by design. Baseline characteristics are presented in **Table 3**. All in all, 29 participants were assessed for eligibility and 23 were randomized (**Figure 2**). Only one participant left during on day 1 of bedrest due to unwillingness to undergo the study protocol and diet. Retention was excellent as 96% of randomized participants completed the full study. All participants who completed the study adhered to the BR and exercise protocol, which will be reported in a forthcoming paper. Two participants left on REC4 and REC5 due to health complications unrelated to the current study, however, they were included in the analysis as all measurements were completed on REC3 and the complications did not confound the results of this study.

3.2 Anthropometrics and Body Composition

Body composition measures were performed before (BDC-3) and after (REC-3) bedrest; results are shown in **Table 4**, with changes illustrated in Figure 3. Total body mass decreased significantly in the exercise (△ pre/post-bedrest [95% CI]; -1.12 kg [-1.80, -0.44] and control (-1.12 kg [-1.59, -0.64]) groups, with no significant differences between groups. The composition of total body mass loss differed between groups, as the exercise group lost significantly more total fat mass (FM) than controls (EX: -0.65 kg [-0.92, -0.38] vs CTL: -0.26 [-0.46, -0.05]; *P* = 0.019), while the loss of total lean soft tissue mass (LSM) was significant in controls (-0.85 [-1.36, -0.33]), but not in the exercise group (-0.48 kg [-1.03, 0.08]). The relative changes (pre/post-bedrest) were not significantly associated with baseline measures of total LSM (*R* = -0.12, *P* = 0.59) and fat mass (*R* = 0.17, *P* = 0.45).

Leg lean mass was assessed since appendicular lean mass may be a better indicator of muscle mass due to the presence of non-contractile lean mass in whole-body measurements; the loss of leg LSM was significant in controls (-0.36 kg [-0.57, -0.15], *P* = 0.004) but not in the exercise group (-0.31 kg [-0.66, 0.04], *P = 0.08*), with no significant difference between groups (*P* = 0.781). Arm and trunk LSM did not change significantly in either group nor differ between groups. Bone mineral content also did not change in either group nor differ between groups.

3.4 Resting energy expenditure

Mean REE (kcal/d) at baseline (B3) and following measurements by indirect calorimetry are shown in **Table 5**. REE was log-transformed and was strongly correlated with LSM at baseline using Pearson's correlation test (r = 0.90, *P* < 0.001) (**Figure 5**). Using repeated-measures ANOVA, an overall period effect was found for log-transformed REE and for REE normalized by LSM (*P* < 0.001), where REE appeared to increase on day 2 and decrease on day 7 of the bedrest period. However post-hoc analysis revealed no significant differences between any given period, nor between groups (**Figure 4**). Correspondingly, LSM changes were not reflected in REE changes (r = -0.15, *P* = 0.49) using Pearson's correlation test. No period nor group effect was found for respiratory quotient (RQ).

3.5 Dietary intake and nutrient adequacy

Mean intake of macronutrients and nutrient adequacy data for all dietary-controlled periods (B, BR, REC) are shown in **Tables 6 and 7**, respectively. Baseline diets were closely matched to the diets provided by the study for energy and macronutrients. Energy intake (kcal/kg) during BR for exercise and control groups were 31 ± 5 and 30 ± 3, respectively. Protein intake (g/kg/d) during BR for exercise and control groups were 1.4 ± 0.1 and 1.3 ± 0.2, respectively. Macronutrient distribution (protein, carbohydrates, fat [% energy]) during the BR period was within AMDR ranges for both exercise and controls without group differences. All group, and group by period interactions were non-significant for macronutrients, fiber, and saturated fat. An overall period effect was found for protein (% of total energy), carbohydrates (% energy), fat (g, % energy), and saturated fat (% fat)- post-hoc analyses revealed an increase in fat intake in the exercise group at recovery when compared to baseline.

No significant period or group effects were found for periodic means of nutrient adequacy that may have an impact on the effect of bedrest and/or exercise on changes in LSM, such as protein, leucine, and n-3 intakes ($P > 0.05$). Protein intake (g/kg BW/d) and N-3 adequacy (% DRI) did not explain the variance in changes in LSM (data not shown). A comparison of mean intake and adequacy for these nutrients are shown as bar plots in **Figure 6**. Pearsons' correlation tests (shown in Table 8) showed that adequate intake of most nutrients was strongly and positively associated with the energy intake (kcal/d), some were moderately correlated (potassium, zinc, manganese, choline) and some were not correlated (n-3, n-6, vitamin A, biotin, vitamin K, and leucine. The use of calcium and vitamin D supplements significantly improved the adequacy of intakes ($P < 0.05$).

Tables and Figures

Table 3: Baseline characteristics

Baseline Parameter	Control (n=11)	Exercise (n=11)
Age, y	59 ± 4	59 ± 3
Sex, male/female, n	5/6	5/6
Height, m	1.67 ± 0.1	1.67 ± 0.8
Body mass, kg	67.6 ± 14.4	72.2 ± 13.3
BMI, kg/m^2	24.0 ± 2.7	25.6 ± 2.9

Table 3. Values are means ± SD; baseline characteristics were determined at screening. BMI = body mass index.

Table 4: Body composition pre- and post-BR

	Control				Exercise				Between-group
	Pre-BR (kg)	**Post-BR** (kg)	**Change** (kg [95% CI])	***Change*** (*P*)	**Pre-BR** (kg)	**Post-BR** (kg)	**Change** (kg [95%CI])	**Change** (*P*)	***Difference*** *(P)*
TBM	67.7 ± 14.7	66.6 ± 14.4	-1.11 [-1.59, -0.64]	< 0.001	72.4 ± 13.5	71.3 ± 13.4	-1.12 [-1.80, -0.44]	0.004	0.986
Total FM	19.3 ± 5.5	19.1 ± 5.5	-0.26 [-0.46, -0.05]	0.021	21.9 ± 5.4	21.2 ± 5.3	-0.65 [-0.92, -0.38]	< 0.001	0.019
Total LSM	45.9 ± 10.7	45.0 ± 10.2	-0.85 [-1.36, -0.33]	0.004	47.8 ± 11.7	47.4 ± 11.7	-0.48 [-1.03, 0.08]	0.085	0.286
Arms LSM	5.3 ± 1.8	5.2 ± 1.9	-0.07 [-0.17, 0.03]	0.157	5.6 ± 2.0	5.6 ± 2.1	-0.04 [-0.16, 0.07]	0.440	0.673
Legs LSM	16.9 ± 4.3	16.6 ± 4.1	-0.36 [-0.57, -0.15]	0.004	17.8 ± 4.0	17.5 ± 3.9	-0.31 [-0.66, 0.04]	0.080	0.781
Trunk LSM	20.5 ± 4.6	20.1 ± 4.1	-0.40 [-0.89, 0.09]	0.099	21.3 ± 5.5	21.1 ± 5.6	-0.19 [-0.69, 0.31]	0.419	0.508
Total BMC	2.5 ± 0.4	2.5 ± 0.4	-0.01 [-0.03, 0.01]	0.245	2.7 ± 0.53	2.7 ± 0.53	0.00 [-0.01, 0.01]	0.904	0.272

Table 4. Values are means ± SD; n = 11 for control group and n = 11 for the exercise group; n = 6 for females and n = 5 for males in each group. Body composition was assessed on B3 (pre-bedrest) and REC3 (post-bedrest). Within-group changes were analyzed using paired t-tests and between-group differences were analyzed by independent t-tests. BR, bedrest; EX, exercise group; CTL, control group; TBM, total body mass; FM, fat mass; LSM, lean soft tissue mass; BMC, bone mineral content.

Table 5: Resting energy expenditure and respiratory quotient pre- and during BR

	Control				Exercise			
	B5	**BR2**	**BR7**	**BR14**	**B5**	**BR2**	**BR7**	**BR14**
REE (kcal/d)	1442 ± 231	1476 ± 256	1418 ± 218	1434 ± 242	1534 ± 291	1609 ± 375	1529 ± 345	1517 ± 305
RQ (CO^2/O^2)	0.82 ± 0.08	0.78 ± 0.08	0.80 ± 0.07	0.81 ± 0.07	0.81 ± 0.07	0.80 ± 0.07	0.77 ± 0.03	0.79 ± 0.04

Table 5. Values are means ± SD; n = 11 (exercise) and n = 11 (control) and measured resting energy expenditure (REE) and respiratory quotient (RQ) before (B5) and during bedrest (BR2, 7, 14). Significant period effect in REE (*P* = 0.008, repeated-measures ANOVA; Tukey's post-hoc analysis revealed no significant differences between time points) whereas none was found for RQ. No group effect nor period by group interaction were found in both REE and RQ. Missing data was imputed by carrying over the last value (n = 5; n = 1 in control and n = 4 in exercise).

Table 6: Average periodic macronutrient intake during dietary-controlled periods

	Baseline		Bedrest		Recovery		Period
Nutrient	CTL	EX	CTL	EX	CTL	EX	*P* value
Energy (kcal)	1954± 386	2140 ± 417	2009 ± 427	2250 ± 528	2013 ± 414	2294 ± 570	ns
(kcal/kg BW)	29 ± 2	30 ± 2	30 ± 3	31 ± 5	30 ± 3	32 ± 5	ns
Protein (g)	87 ± 16	96 ± 17	88 ± 18	96 ± 17	84 ± 16	98 ± 17	ns
(g/kg BW)	1.3 ± 0.1	1.3 ± 0.1	1.3 ± 0.2	1.4 ± 0.1	1.3 ± 0.1	1.4 ± 0.2	ns
(% energy)	17.8 ± 1.8	18.1 ± 1.2	17.5 ± 1.3	17.3 ± 1.3	16.8 ± 1.2	17.4 ± 1.8	*
Carbohydrates (g)	250 ± 50	272 ± 53	256 ± 55	286 ± 68	248 ± 49	272 ± 77	ns
(% energy)	50.8 ± 3.0	51.0 + 3.0	51 ± 2.8	50.9 ± 3.1	49.5 ± 4.7	47.1 ± 4.6	**
Fiber (%DRI)	123 ± 23	139 ± 24	124 ± 24	141 ± 17	125 ± 29	132 ± 29	ns
Fat (g)	75 ± 16	79 ± 17[a]	77 ± 18	88 ± 25	81 ± 22	96 ± 26[a]	**[gg]
(% energy)	34.1 ± 2.3	33.2 ± 2.1[b]	34.4 ± 3.3	34.8 ± 3.6[c]	36.2 ± 4.7	37.8 ± 3.9[b,c]	***
Saturated fat (% fat)	10.4 ± 1.4	10.5 ± 1.0	10.6 ± 1.7	10.9 ± 1.2	11.0 ± 1.9	11.9 ± 1.7	*

Table 6. Values are mean ± SD; n = 11 for control group (CTL) and n = 11 for the exercise group (EX). Macronutrient intakes by period was reported as % of total energy intake. All group, and group by period interactions were non-significant (*P* > 0.05). Overall period effects were found for protein (% energy), carbohydrates (% energy), fat (g, % energy) , and saturated fat. Significant post-hoc *P*-values (< 0.05) for within groups period effects are denoted with superscript and Greenhouse-Geisser corrections were made for departures from sphericity: [gg]). Significant levels for group and period effects (ns: *P* > 0.05, *: *P* < 0.05, **: *P* < 0.01, ***: *P* < 0.001). Baseline period (5 days); bedrest period (14 days); recovery period (7 days); BW, body

Table 7: Average periodic nutrient adequacy ratio (% of DRI) during dietary-controlled periods

	Baseline		**Bedrest**		**Recovery**		**Group**	**Period**
Nutrients, % of DRI	CTL	EX	CTL	EX	CTL	EX	*P value*	
N-3	118 ± 80	94 ± 33	124 ± 76	134 ± 54	126 ± 83	117 ± 39	ns	ns
N-6	64 ± 27	59 ± 25	66 ± 26	68 ± 28	65 ± 20	70 ± 22	ns	ns
Calcium	85 ± 31	98 ± 38	98 ± 30	105 ± 30	87 ± 28	95 ± 32	ns	*
with supplements	196 ± 38	207 ± 48	208 ± 38	215 ± 40	197 ± 37	204 ± 42	ns	*
Iron	169 ± 27	180 ± 33	176 ± 30	190 ± 40	167 ± 27	181 ± 38	ns	ns
Magnesium	95 ± 10	104 ± 15	103 ± 17	114 ± 18	99 ± 14	111 ± 14	ns	*
Phosphorus	176 ± 35	194 ± 48	188 ± 39	209 ± 50	187 ± 36	210 ± 47	ns	*
Potassium	103 ± 12	122 ± 18	113 ± 17	119 ± 12	106 ± 15	116 ± 15	ns	*
Sodium	173 ± 42	191 ± 54	159 ± 42	174 ± 36	166 ± 44	186 ± 48	ns	ns
Zinc	94 ± 10[a]	103 ± 14	100 ± 17	111 ± 14	93 ± 12[b]	114 ± 15[a,b]	**	ns
Copper	220 ± 25	231 ± 56	233 ± 40	250 ± 74	226 ± 46	247 ± 71	ns	ns
Manganese	201 ± 33	199 ± 25[c]	216 ± 41	246 ± 44[c]	198 ± 46	225 ± 38	ns	***
Selenium	159 ± 39	167 ± 34	158 ± 24	173 ± 37	165 ± 36	191 ± 49	ns	ns
Vitamin A	118 ± 23	143 ± 33	122 ± 20	121 ± 24	120 ± 28	137 ± 21	ns	ns
Thiamin	96 ± 18	109 ± 21	97 ± 17	113 ± 20	100 ± 17	105 ± 25	ns	ns
Riboflavin	132 ± 27	164 ± 31	148 ± 33	166 ± 28	138 ± 23	165 ± 30	*	ns
Total niacin equivalents	199 ± 34	235 ± 41	216 ± 29	228 ± 36	211 ± 33	246 ± 46	ns	ns
Pantothenic acid	101 ± 17	118 ± 29	110 ± 23	121 ± 32	105 ± 19	119 ± 35	ns	ns
Vitamin B6	98 ± 15[d]	111 ± 21	113 ± 17[d]	117 ± 20	108 ± 17	111 ± 22	ns	**
Biotin	2.6 ± 2.5	2.5 ± 2.0	4.4 ± 6.4	4.3 ± 3.3	2.8 ± 2.7	2.3 ± 1.8	ns	ns
Folate	136 ± 29	148 ± 35	135 ± 18	144 ± 35	138 ± 22	143 ± 34	ns	ns
Vitamin B12	114 ± 28	130 ± 34	124 ± 31	138 ± 32	106 ± 28	142 ± 45	ns	ns
Choline	50 ± 9.7[e]	62 ± 15	51 ± 9.7[f]	63 ± 14	58 ± 12	70 ± 15[e,f]	*	**
Vitamin C	182 ± 51	225 ± 66	203 ± 39	205 ± 41	194 ± 67	200 ± 46	ns	ns

Table 7: Average periodic nutrient adequacy ratio (% of DRI) during dietary-controlled periods

	Baseline		**Bedrest**		**Recovery**		**Group**	**Period**
Nutrients, % of DRI	CTL	EX	CTL	EX	CTL	EX	*P value*	
Vitamin D	20 ± 6	25 ± 10	26 ± 12	27 ± 6	24 ± 12	30 ± 12	ns	ns
with supplements	187 ± 6	191 ± 10	193 ± 12	194 ± 6	191 ± 12	197 ± 12	ns	ns
Vitamin E	57 ± 14	63 ± 18	62 ± 14	69 ± 23	63 ± 15	71 ± 20	ns	ns
Vitamin K	281 ± 89	278 ± 76	284 ± 82	255 ± 83	259 ± 86	279 ± 68	ns	ns
Leucine	103 ± 13	114 ± 13	100 ± 15	103 ± 13	92 ± 26	107 ± 20	ns	ns

Table 7. Values are mean ± SD; n = 11 for control group (CTL) and n = 11 for the exercise group (EX). All nutrient values are expressed as a % of DRI (except leucine), determined by age and sex categories. Nutrients for which Recommended Daily Allowance (RDA) exist are in **bold**, others were evaluated against Adequate Intake (AI) levels. All group by period interactions were non-significant ($P > 0.05$, repeated-measures ANOVA). Significant levels for group and period effects (ns: $P > 0.05$, *: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$). Significant post-hoc *P*-values ($P < 0.05$) for within group period effects are denoted with superscript ([gg]: Greenhouse-Geisser corrections for departures from sphericity). N-3, omega-3 as a sum of alpha-linolenic acid, decohexanoic acid, and eicosonoic acid; N-6, omega-6 or gamma-linolenic acid; Folate, dietary folate equivalents (DFE); Vitamin A, total retinol activity; Vitamin E, alpha-tocopherol. B, baseline period (5 days); BR, head-down tilt bedrest period

Table 8: Correlation matrix between nutrient adequacy and energy intake

	Energy, kcal/d	
Nutrient, % DRI	*r*	*P*
N-3	0.06	ns
N-6	0.25	ns
Calcium	0.93	***
Iron	0.95	***
Magnesium	0.74	***
Phosphorus	0.96	***
Potassium	0.57	**
Sodium	0.80	***
Zinc	0.69	**
Copper	0.92	***
Manganese	0.62	**
Selenium	0.84	***
Vitamin A	0.22	ns
Thiamin	0.95	***
Riboflavin	0.89	***
Niacin	0.92	***
Pantothenic acid	0.90	***
Vitamin B6	0.81	***
Biotin	0.34	ns
Folate	0.78	***
Vitamin B12	0.78	***
Choline	0.44	*
Vitamin C	0.65	***
Vitamin D	0.63	**
Vitamin E	0.80	***
Vitamin K	-0.17	ns
Leucine	0.12	ns

Table 8. Pearson's correlation coefficients (*r*) are given for each nutrient's association with energy intake, with **bold type** describing those with significant p-values (*P*). Significant associations (*: P <0.05, **: P <0.01, ***: P <0.001). Non-significant associations (P > 0.05).

Figure 2: CONSORT diagram

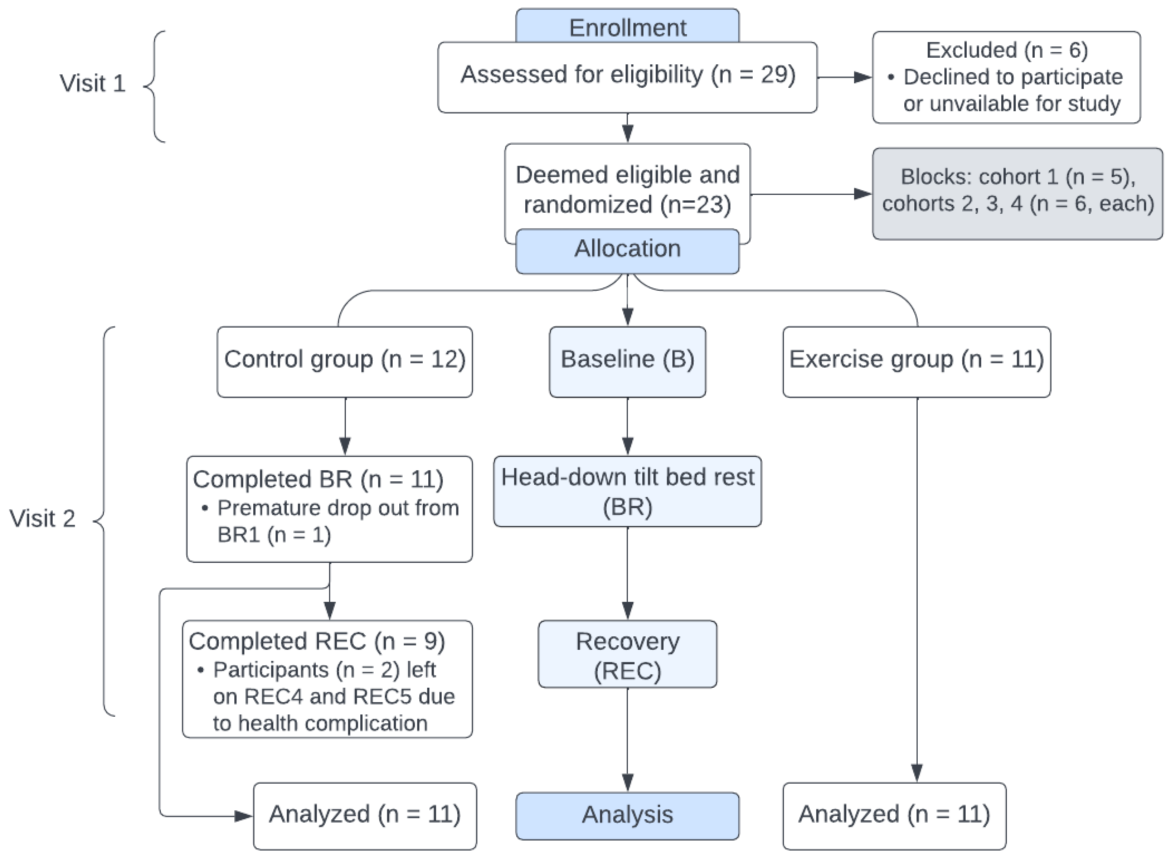

Figure 3: Changes in body composition from pre to post-14 days of bedrest

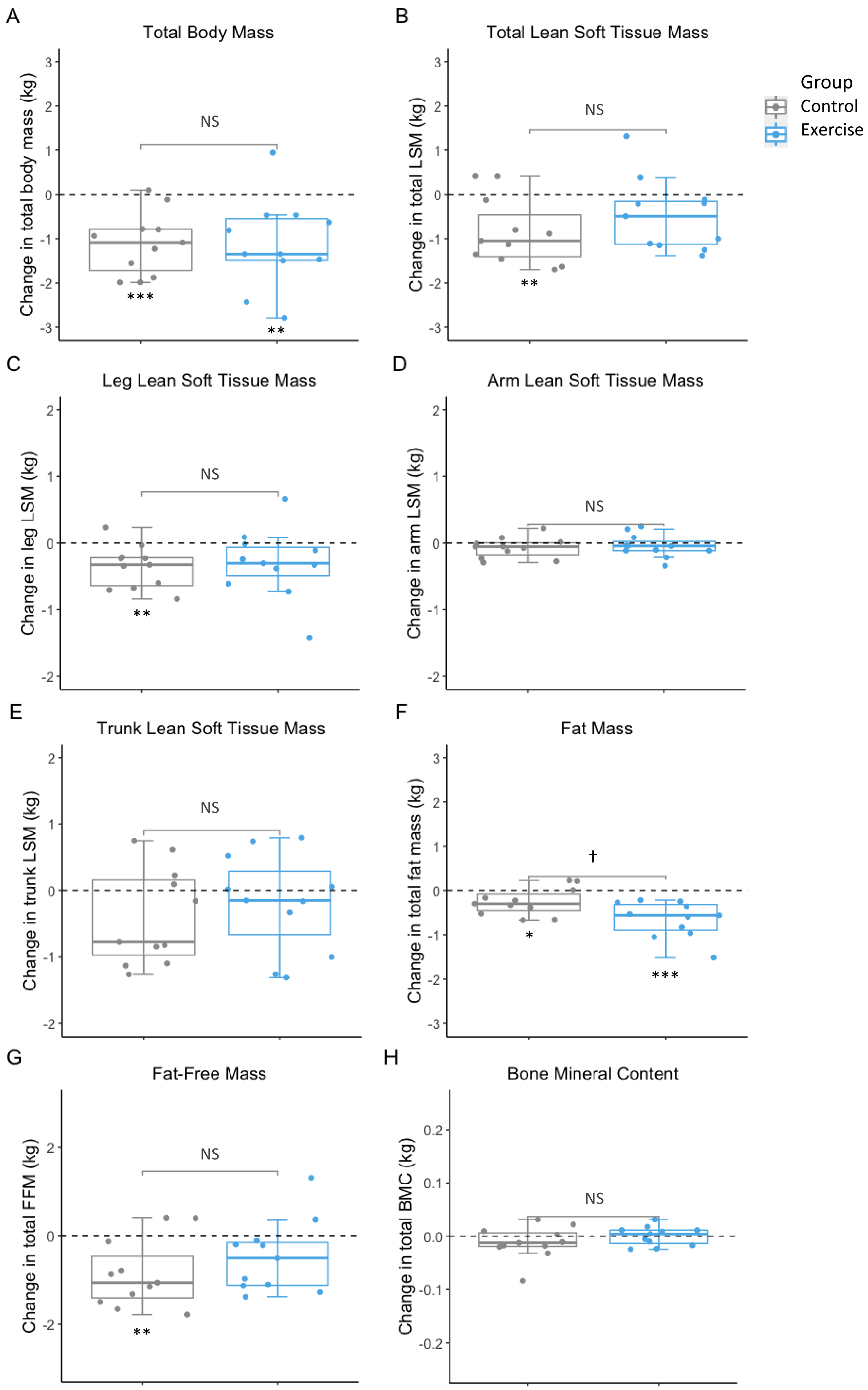

Figure 3. Box plots represent median, interquartile ranges (IQR), and 1.5*IQR as whiskers in control (n = 11) and exercise (n = 11) groups. Significant change pre-/post- 14-d bedrest (*: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$, paired t-test). Significant difference between groups (†: $P < 0.05$, independent t-test).

Figure 4: Resting energy expenditure pre- and during bedrest period

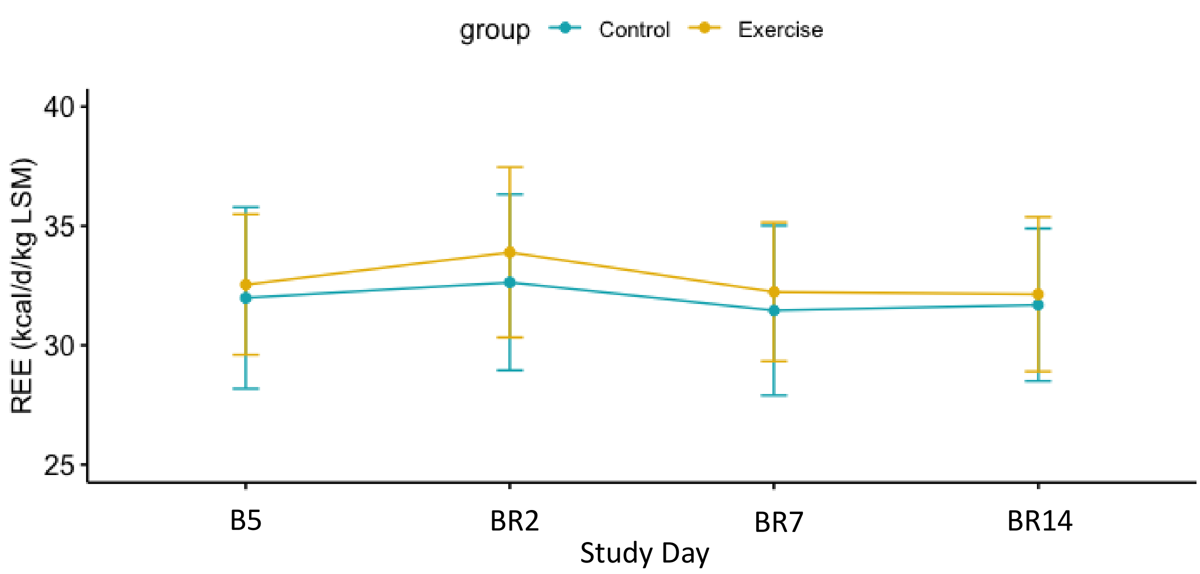

Figure 4. Values are means ± SD of REE normalized by baseline LSM in control (n = 11) and exercise (n = 11) groups. Repeated-measures ANOVA: significant period effect ($P < 0.001$), no significant group effect, and Tukey's post-hoc analysis revealed no significant differences between time points. The same findings were true of absolute REE which was log-transformed (period effect, $P = 0.001$). Missing data was imputed by carrying over the last value (n = 5; n = 1 in control and n = 4 in exercise).

Figure 5: Correlation between baseline resting energy expenditure and total lean mass

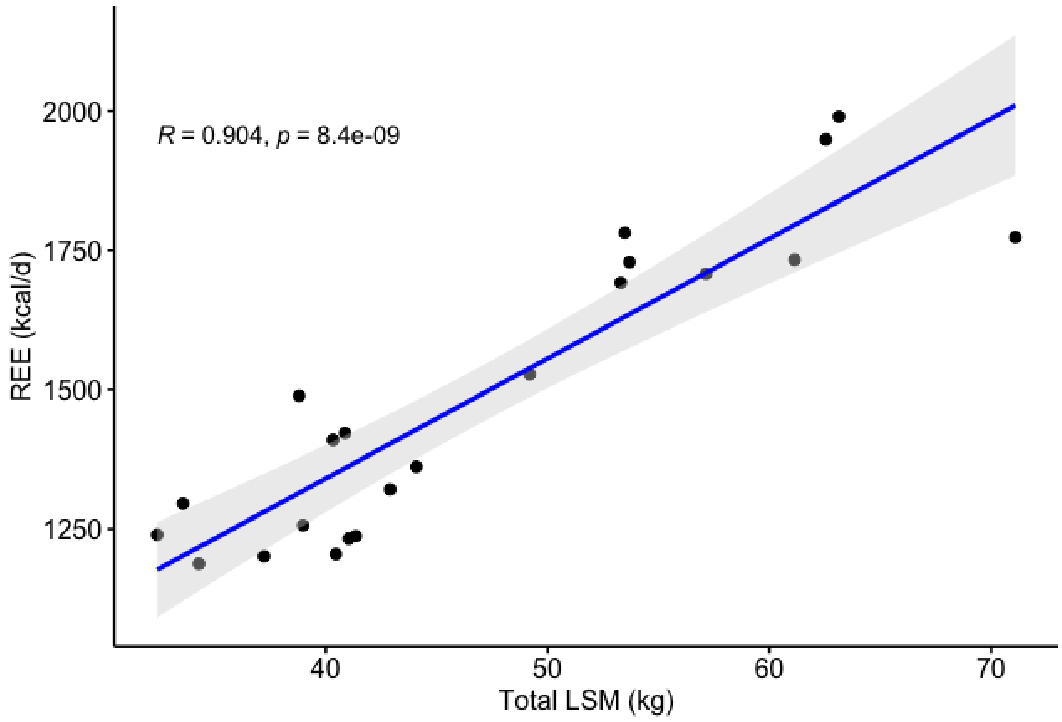

Figure 5. Pearson's correlation plot for resting energy expenditure (REE) and total lean soft tissue mass (LSM) at baseline for control (n = 11) and exercise (n = 11) groups. LSM was strongly associated with REE at baseline, independent of total body weight and sex (R = 0.90, *P* < 0.001).

Figure 6: Bar plots of nutrient intakes and adequacy during dietary-controlled periods

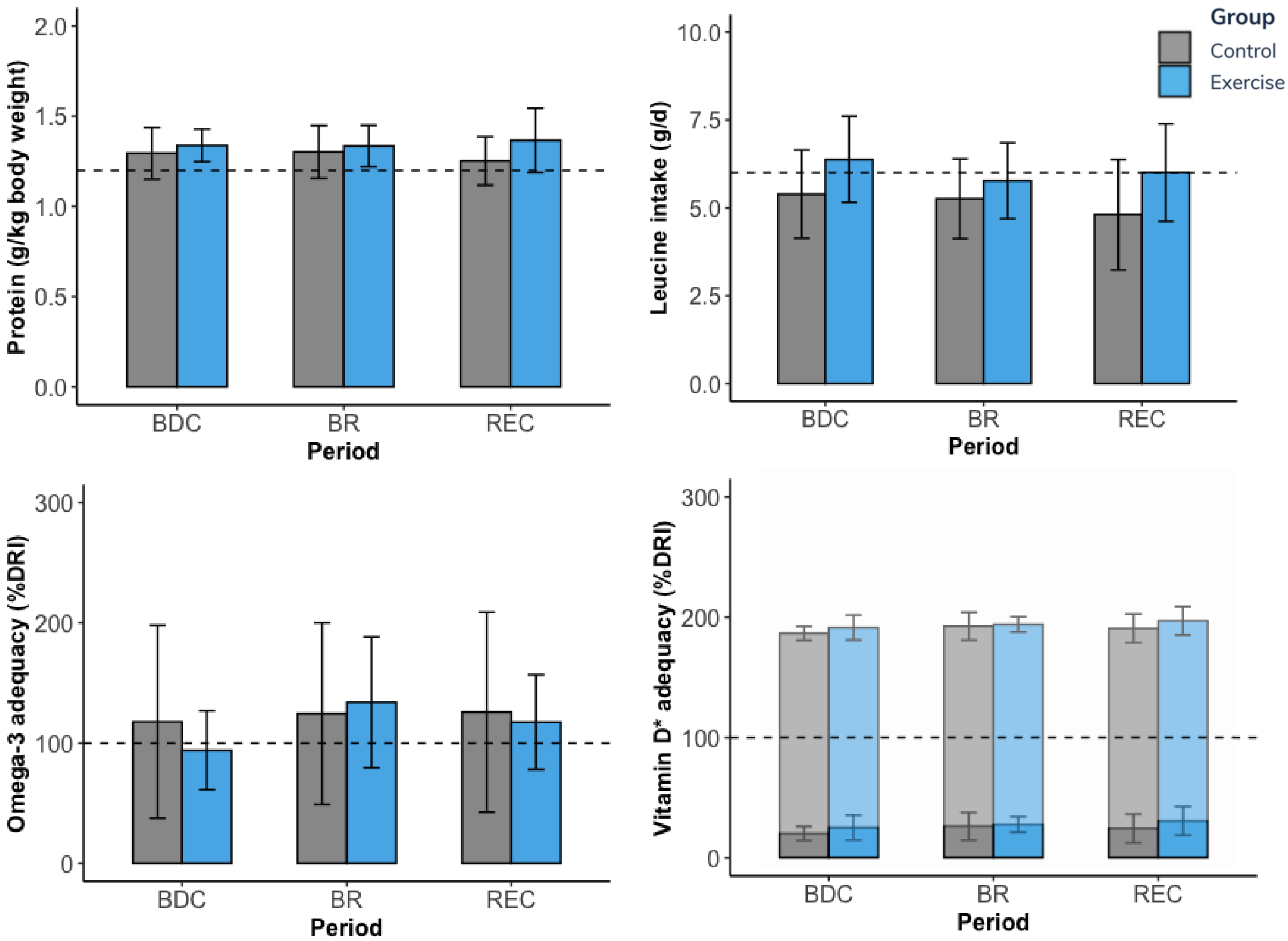

Figure 6. Plots represent mean ± error bars (SD) for control (n = 11) and exercise (n = 11) groups. All nutrient intake values are expressed as a % of DRI or dietary targets (*: transparent bars represent intake with supplements). All group by period interactions were non-significant (*P* > 0.05, repeated-measures ANOVA). n-3, omega-3 as a sum of alpha-linolenic acid, decohexanoic acid, and eicosonoic acid; B, baseline period (5 days); BR, head-down tilt bedrest period (14 days); REC, recovery period (7 days).

Chapter 3: Discussion

Microgravity and inactivity mechanically unload the musculoskeletal system which inevitably lead to muscle atrophy without the use of countermeasures, such as exercise. Muscle atrophy and associated losses of strength are key features of clinical sarcopenia and spaceflight, which may drastically impair an individual's quality of life and autonomy. In this study, we sought to determine the efficacy of a time-efficient (1 hour/day) multimodal exercise countermeasure in preventing muscle atrophy among bedridden healthy older adults. As expected, 14 days of head-down tilt bedrest (HDBR) caused a modest loss of total-body lean soft tissue mass in the non-exercising group. Despite prior evidence which suggesting the presence of anabolic resistance due to ageing and muscle disuse [91], exercise attenuated the loss of lean mass and promoted greater fat loss despite comparable weight loss among both groups. We also hypothesized that resting energy expenditure would follow the decrease in metabolically active lean soft tissue mass. However, resting energy expenditure did not change. Participants' overall nutrient intake was deemed adequate given the highly controlled and carefully planned menu. The retention rate was excellent, as only one individual withdrew during the first day of bedrest. The low time commitment of the exercise countermeasure and careful supervision from the exercise staff may have contributed to the high retention rate and adherence to the protocol.

3.1 The impact of exercise on changes body composition among older adults undergoing HDBR

This bedrest study demonstrated a partially protective effect of exercise on the anticipated loss of lean mass in healthy older adults. Both groups experienced a significant but modest weight loss in both groups, though 14 days of bedrest resulted in a -1.2% loss of total-body lean soft tissue mass in the control group, while the multi-modal exercise protocol attenuated this loss and promoted a significantly greater loss of fat mass in older adults. Consistent with previous bedrest studies, muscles functioning for posture or anti-gravitational purposes were impacted to a greater degree than others, such that the loss of lean mass in the control group was primarily explained by changes in the legs and no changes were detected in the trunk or arm regions [92]. As total-body measures include non-contractile lean soft tissue, leg lean mass may also represent a better estimate for limb skeletal muscle mass. Therefore, one hour/day of the proposed multi-modal exercise countermeasure may be sufficient to preserve muscle mass in older adults undergoing bedrest, given an overall adequate nutrient intake. Although these results may not be directly applicable to patients with illnesses, the findings provide new evidence regarding healthy older adults' tolerance and metabolic response to exercise countermeasures during bedrest, without the extraneous factors associated with hospitalization or spaceflight. It is likely that older adults with illness or in microgravity would experience an even greater loss of lean tissue mass during the same period of time [21, 93].

Since the between-group differences in changes of total and leg lean mass loss were non-significant, the efficacy of exercise in mitigating muscle atrophy during bedrest cannot be firmly concluded. The low sample size and inadequate power may explain the lack of group differences. There are currently no bedrest studies of moderate duration (≥ 14-d) with older adults, undergoing concomitant exercise, limiting comparison of our findings. Head-down tilt-induced fluid shifts have also been demonstrated to lead to diuresis [94], however, fluid balance was not measured and consequently could not be excluded as a confounder to the observed changes in body weight and lean soft tissue mass during the 14-d BR period. Some studies have suggested that older adults are more susceptible to muscle mass loss than their younger counterparts due to differences in baseline fat-free mass and anabolic sensitivity [95]. However, in our study, changes in lean mass were not significantly associated with baseline values when tested by Pearson's correlation. It is impossible to determine the extent to which anabolic resistance may have diminished the response to exercise and adequacy of key nutrients due to the absence of a younger comparison group. Regardless, the loss of lean mass observed among the control group was modest and appeared to be mitigated with the exercise countermeasure.

Reduced physical activity is considered a risk factor for metabolic syndrome as it can result, among others, in the accumulation of adipose tissue in healthy adults due to the blunting of triacylglycerol catabolism and fatty acid oxidation, which are partially mediated by skeletal muscle tissue [96]. Intermuscular adipose tissue has also been associated with lower muscle strength and physical performance [40, 97, 98], though DXA scans are not capable of assessing fat infiltration into muscle tissue. Our study demonstrated that total fat mass loss was greater in the exercise group despite similar weight loss between groups, suggesting a preferential use of fat substrates and increased oxygen consumption with the higher-intensity components of the exercise countermeasure. However, the respiratory quotient did not differ between groups, and results suggest a mixed utilization of substrates (RQ ~0.8) rather than a greater use of fat (~0.69) [99]. The lack of group differences contrasted with previous findings by Paoli et al [10] who demonstrated the effect of high-intensity resistance training (HIRT) on promoting fat loss with a corresponding decrease in RQ (before exercise: 0.827 ± 0.006, 22 h post-exercise: 0.798 ± 0.010) when compared to traditional resistance training (22 h post-exercise: 0.822 ± 0.08). Although our study included aerobic and HIIT training, which typically require more oxygen consumption, the greater length of time between training sessions and indirect calorimetry tests which may have diminished the effect of exercise on measured RQ. Further studies are required to understand the link between exercise intensity and substrate utilization during bedrest in ameliorating muscle mass and strength outcomes.

3.2 Resting energy expenditure during bedrest

Resting energy expenditure is a major component of total energy expenditure and is primarily determined by the presence of metabolically active tissue [100]. Accordingly, LSM was strongly associated with REE at baseline, independent of fat mass, weight, and sex. Therefore, changes in REE were expected to follow changes in LSM, but associations were non-significant. The discrepancy may be explained by the lack of change in trunk LSM, as organs of high metabolic rate are primarily located in this region -and contribute importantly to REE- but comprise a minor portion of total LSM [45, 100]. Gretebeck et al. [101] were the first to characterize TEE during a 10-d BR study using doubly-labelled water and observed a 21% decrease in TEE when compared to an ambulatory control group, but no changes in REE occurred; in contrast to this study, there was a significant increase in FM in the younger population (n = 9; age: 35.8 ± 4.6 y) and no changes in FFM were reported.

An overall period effect was found for REE which appeared to increase on BR2 and to drop below baseline values on BR7 but could not be confirmed due to insignificant post-hoc analyses. The lack of a clear trend in REE was surprising as an increase was expected in the exercise group due to excess post-oxygen consumption (EPOC) associated with HIIT [56]. Upon further evaluation, this could be explained by the performance of HIIT one day prior to REE measurements on BR2 and BR14, but more than one day prior to BR7 (the exercise schedule has been detailed by Hedge et al. [27]). These findings may corroborate with findings by Paoli et al. [10] who demonstrated a greater increase in REE (measured 22 hours post-exercise) from high-intensity interval resistance (HIRT) when compared to traditional resistance training ($P < 0.001$) among healthy and active young men (n = 18, age = 28 ± 4.5 years). Some studies evaluating the effects of exercise on energy balance in sedentary participants have also observed that REE adapts to body weight changes in an effort to minimize energy deficits [102]; however, the weight loss exhibited in our study was modest and the duration of the study may have been too short to provide such evidence. As knowledge of energy requirements is required to achieve energy balance, longer duration studies with a greater sample size are required to determine older adults' response to a similar multi-modal exercise protocol on individual components of energy expenditure.

3.3 Nutritional factors implicated in the maintenance of lean mass during HDBR

Energy deficits can occur due to increased energy expenditure and/or decreased energy intake. Although a balanced and isocaloric diet was provided, participants were given the liberty to determine their level of fullness and stop eating when they were satisfied. Most participants described difficulties with eating the prescribed amounts of food during the BR period, such as eating in a prone position, and loss of appetite related to headaches, gastrointestinal discomfort, and lower non-exercise physical activity. Therefore, many participants refused extra meals or snacks and were unable to maintain energy balance, resulting in weight and fat mass loss. Previous studies have also shown that exercise does not stimulate an increase in spontaneous energy intake [103-105]. Accordingly, energy intake did not differ between groups, which likely contributed to an energy deficit among the exercise group. Despite efforts to mitigate weight changes in individuals by increasing their prescribed energy intake, modest weight loss persisted in both groups which may be attributed to positional, psychological, or behavioral factors associated with bedrest. Notably, the study's diet was carefully designed to ensure acceptability and adequate control of nutrient intake among participants which may not be realistic to spaceflight or hospital environments. Therefore, older adults undergoing longer duration spaceflight or bedrest would likely experience an even more important loss of total body mass.

Protein, omega-3 fatty acids, vitamin D, and leucine intakes have been implicated in the maintenance of lean mass during states of energy balance [47], and especially in energy deficit [49]. Despite providing a diet that met or exceeded the age- and sex-specific DRI's for protein, omega-3 fatty acids, vitamin D (with supplementation), and leucine, muscle loss was significant in the control group. However, the magnitude of this loss was relatively small when compared to previous bedrest studies including older adults. Kortebein et al. [4] used DXA to measure changes in total lean mass following a 10 days of bedrest among healthy older adults (n= 10, age: 67 ± 5 y; women: 50%; BMI: 29 ± 3 kg/m^2) and observed a change of -1.5 kg [95% CI: -2.48, -0.62], equivalent to -3.2% relative to pre-bedrest measures. Compared to our control group (post-BR change: -1.7%), the greater loss of total lean mass could be attributed to the fact that participants were in a negative nitrogen balance with a diet providing 0.8 g/kg/d of protein which is substantially lower than this study's provision of protein (1.2 g/kg/d). This would corroborate with previous suggestions that protein intakes beyond the current RDA (0.8 g/kg/d) may assist in the maintenance of lean mass in older adults. Energy intake was related to nutrient adequacy, which is an important element of optimizing the anabolic response to exercise countermeasures during prolonged bedrest [66]. Spontaneous dietary intake could not be assessed within the semi controlled-feeding trial, however, this study showed that it is possible for bedridden participants to attain adequate intake of most nutrients with careful menu planning and nutrition counselling.

3.4 Strengths

To our knowledge, this is the first study to implement an exercise countermeasure among older men and women undergoing two weeks of head-down tilt bedrest. The use of gold standard methods, such as DXA and indirect calorimetry, allowed for a reliable assessment of body composition and REE. The time commitment for the exercise countermeasure (1 hour/day) was notably shorter than the current protocols recommended by space agencies (2.5 hours/day) due to the inclusion of HIIT; further, the 100% retention rate among the exercise group indicates that older men and women are able to tolerate the intervention. Energy expenditure and body composition were also measured using gold standard methods, indirect calorimetry and DXA imaging, providing a reliable assessment of changes in body composition and resting energy expenditure. Numerous strengths pertain to the study diet in efforts to minimize its potential effects on the exercise intervention and study outcomes- All foods and beverages were provided for consumption onsite with attention to important design aspects of controlled-feeding trials [106]; estimation of energy requirements with the use of indirect calorimetry, standards for diet composition, nutrient adequacy targets, menu rotation and options to avoid food boredom or nonadherence, regular monitoring of body weight, and a 5-d run-in period. The use of an image-based AI food tracking software (Keenoa) allowed for rapid and continuous assessment of individual food intake during the study to promote nutrient adequacy within participants. Finally, the presence of nutrition staff 16 hours/day to prepare meals and assess nutrient intake allowed for excellent dietary control and likely contributed to the high retention rate.

3.5 Limitations

The larger Inactivity Study was a highly complex and integrated bedrest study that involved measurements from multiple teams across Canada. A larger sample size would be useful to achieve adequate power to detect significant interaction and subgroup effects (i.e., group by period and sex effects). However, increasing the number of participants was challenging due to the physical and social demands of the study, as well as high organizational demands of bedrest studies; as such, an important weakness of all bedrest studies is their small sample size [47]. Also, the preferred testing day for post-BR measurements would be the first day that participants got out of bed (REC1), not all measurements could be conducted on this day due to time limitations and participant fatigue considerations. Consequently, post-BR measurements of body composition was completed on REC3 and may underestimate the magnitude of changes attributed to 14-d bedrest. Additionally, though DXA provides accurate measurements to determine body composition, the precision of measurements (± 310 g LSM) may not reliably meet the threshold to detect modest changes of whole-body measures such as total LSM. DXA is unable to differentiate fluids within lean mass, therefore, the effect of diuresis associated with the head-down tilt-induced fluid shifts could not be excluded as a confounder to the observed changes in body weight and lean soft tissue mass during the 14-d BR period. Since both groups experienced a similar and modest weight loss, it is likely that energy balance would have improved lean soft mass outcomes in both groups. Although a controlled diet was provided to maintain weight and nutrient adequacy throughout the study, a certain amount of flexibility was given to ensure the comfort of participants. This flexibility has the benefit of being realistic to spaceflight and hospitalized environments but may have contributed to the unintended weight loss observed.

3.6 Future directions

The planning of longer space missions to achieve interplanetary travel requires astronauts to sustain sufficient energy intake to ensure nutritional adequacy and optimize the likelihood of mission success. Although the evaluation of individual countermeasures is important to measure its efficacy among specific populations, it has been well-documented that individual strategies (exercise or nutrition alone) are insufficient for the maintenance of muscle mass during prolonged bedrest and spaceflight. While exercise attenuated the loss of total and leg lean soft mass in the intervention group, there was no between-group difference, while the presence of overall weight loss may partially explain the small effect size. Notably, the high-quality meals provided in this study were optimized for participant enjoyment and included many fresh components- this may not be realistic to reproduce in spaceflight and hospital settings. Therefore, future trials should explore the use of high-energy and protein nutritional supplements to potentiate the effect of a similar exercise countermeasure during BR among healthy older adults.

This study showed large interindividual differences for REE. The development of devices to measure components of energy expenditure in real-time would help to determine individual requirements for astronauts during long-duration spaceflight to maintain weight and lean mass. Findings would also support the planning of future space food systems to provide adequate nutrients to the crew. While previous prolonged BR studies have suggested that single-nutrient supplementation alone may not attenuate the loss of lean mass, further work is needed to confirm the efficacy of leucine or n-3 supplementation during unloading, and particularly its interaction with exercise. Since exercise is known to have a potentiating effect on nutritional stimuli, a novel controlled-feeding bedrest RCT may evaluate muscle maintenance in a multi-modal intervention group performing regular exercise with n-3 supplementation, compared to another exercise group without supplementation. To validate the effectiveness of such an intervention in space, a similar study may be conducted on astronauts before and after a long-duration mission.

Bedrest studies enable the study of microgravity and potential countermeasures to improve the safety of space flight on humans. There are important terrestrial applications of these studies, such as to observe the effects of inactivity on the trajectory of muscle loss, and to promote exercise and nutrient adequacy among an increasingly sedentary global population. Importantly, the lower projected cost of spaceflight in the medium-term future will enable the study of the physiology of ageing in an accelerated manner. The mutual benefit between terrestrial and space health-related research is the rationale behind the development of the study's research umbrella- *Microgravity Research Analogue: Understanding the Health Impact of Inactivity for the Benefit of Older Adults and Astronauts*. Collaborative efforts to link the research interests related to space exploration and healthy aging will serve to enrich our knowledge on longevity, benefitting astronauts and civilians on earth.

Chapter 4: Conclusion & Significance

4.1 Conclusion

This RCT is the first to assess the impact of 14-d head-down tilt bedrest and a concomitant exercise countermeasure among healthy older adults (55-65 yo). Using DXA, indirect calorimetry, and an artificial intelligence, image-based food tracking software (Keenoa), this sub-study characterized differential changes in body composition, resting energy expenditure, and nutrient adequacy. As expected, the non-exercising group experienced a significant amount of total and leg lean soft tissue loss, which was attenuated by a time-efficient exercise protocol incorporating HIIT. Consistent with previous studies, bedrest induced a significant but small weight loss among both groups, which was primarily attributed to lean soft mass loss in the control group, and to fat mass loss in the exercise group.

Weight loss may have also been impacted by HDBR-related challenges to overall intake, such as eating position, low non-exercise physical activity, and various discomforts associated with the head-down tilt-induced headward fluid shift. Contrary to our expectations, REE did not follow changes in lean mass and was not affected by exercise. It was feasible to provide adequate levels of most nutrients despite the challenges to overall intake. Further, controlling for intakes of key nutrients involved in the upregulation of muscle protein synthesis did not explain the variance in changes of lean mass within either group. Future trials should explore the use of high-energy nutritional supplements during BR in older adults to improve the efficacy of exercise countermeasures during mechanical unloading. Validation of devices that can provide instant assessment of body composition and components of energy expenditure would enable rapid intervention in both clinical and spaceflight environments.

4.2 Significance

Bedrest is commonly indicated during hospitalization, where pre-habilitation and rehabilitation are not frequently implemented. Inactivity is also pervasive throughout population of older adults and astronauts, such that exercise recommendations are seldom followed due to perceived time constraints. The exercise countermeasure evaluated in this study led to greater fat loss while eliciting a shorter time commitment and may help reduce the barrier to exercise. The study demonstrated an exceptionally low attrition rate (4% overall, 0% in exercise group), which points to the feasibility of this multi-modal exercise countermeasure in healthy older adults. It should be noted that the current study may not be totally applicable to patients in-hospital with additional stressors, such as certain diseases or severe injury, the intensity of exercise required to attenuate muscle atrophy may not be feasible.

The current study provides novel evidence suggesting the efficacy of 1 h/d of a multi-modal exercise countermeasure (aerobic, HIIT, and resistance training) in attenuating the loss of lean mass among older adults undergoing 14-d BR. Although weight loss was comparable and the difference between LSM changes between groups was not statistically significant, two weeks of BR conferred a -0.6 kg mean difference in the loss of LSM between the exercise and control groups, which represents the change that is expected over a year of normal age-related muscle loss. It is important to note that relative declines in muscle strength often exceed that of muscle mass, which is an arguably a better indicator of functional capacity. However, since muscle mass (estimated by LSM) is an important predictor of muscle strength and function, modest changes in LSM may confer a real impact on the functional capacity of older adults who experience greater difficulty in recovering from the loss compared to their younger counterparts [107].

A large interindividual variation was found for REE in both groups, which coincides with findings from a recent study among exercising astronauts undergoing ≥ 3 months of spaceflight. This signifies the need for real-time measurements of components of energy balance to individualize inflight caloric recommendations. Also, a very high-quality diet was maintained and contributed to overall nutrient adequacy which may not be realistic in the context spaceflight or hospitalization. Therefore, multi-modal (exercise and nutrient) countermeasures should be explored further.

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Appendix A: Supplemental methods for Manuscript

Table 1. Recommended levels of nutrient intake

	CSA, AI	**IOM, DRI (51-70 y)**	
Nutrient	**Both sexes**	**Men**	**Women**
Energy and Macronutrients			
Total fat (%TEE)	30-35	20-35	20-35
Saturated fatty acids (%TEE)	≤ 10	-	-
Monounsaturated fatty acids (%TEE)	≥ 10	-	-
Polyunsaturated fatty acids (%TEE)	≥7	-	-
N-3	-	1.6	1.1
N-6	-	11	14
Protein	1.2 g/kgBW/d	10-35 %TEE	10-35 %TEE
Carbohydrates (%TEE)	50-60	45-65	45-65
Total fiber (g/d)	≥ 30	30	21
Electrolytes and Water			
Sodium (g/d)	3.5 to 4.5	1500	1500
Chloride (g/d)	6.0 to 7.5	-	-
Potassium (g/d)	3.5 to 5.0	3400	2600
Calcium (mg/d)	1000-1200	**1000**	**1200**
Water (ml/kgBW/d)	100	-	-
Vitamins			
Biotin (ug/d)	5	30	30
Pantothenic acid (mg/d)	400	5	5
Folate (ug/d)	20	**300**	**300**
Niacin (mg/d)	1.5	**16**	**14**
Thiamin (mg/d)	1.5	**1.2**	**1.1**
Vitamin B6 (mg/d)	2	**1.7**	**1.6**
Vitamin B12 (ug/d)	2	**2.4**	**2.4**
Vitamin K (ug/d)	80	120	90
Vitamin D (ug/d)	5	**15**	**15**
Vitamin A (ug/d)	1000	**900**	**700**
Vitamin C (mg/d)	100	**90**	**90**
Vitamin E (mg/d)	20	**15**	**15**
Elements			
Copper	1500-3000 ug/d	**0.7 mg/d**	**0.7 mg/d**
Fluoride (mg/d)	1.5-4	-	-
Iodine (ug/d)	200	-	-
Iron (mg/d)	10	**8**	8
Magnesium (mg/d)	300	**420**	**320**
Phosphorus (mg/d)	700-1500	**700**	**700**
Zinc (mg/d)	-	**11**	**8**

Appendix A. Table 1. Values are listed as recommended daily allowance (RDA) in **Bold** when available, or else adequate intake (AI) are given. Macronutrients are listed as Acceptable Macronutrient Distribution Ranges (AMDR, % TEE). CSA, Canadian Space Agency; IOM, Institute of Medicine; DRI, dietary reference intakes. CSA's AI levels were used to determine the minimum level of intake for menu formulation during dietary-controlled periods. DRI levels are listed if used in the analysis of nutrient adequacy, according to age and sex.