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Effects of exercise therapy combined with dietary supplements on the loss of skeletal muscle mass during bed rest - a systematic review.

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Dissertação de candidatura ao grau de Mestre em Medicina, submetida ao Instituto de Ciências Biomédicas Abel Salazar – Universidade do Porto

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RESUMO

INTRODUÇÃO

A perda de massa muscular esquelética durante o voo espacial é um grande desafio para a Medicina na exploração espacial. A microgravidade encontrada no ambiente espacial é responsável pela disrupção de vários sistemas fisiológicos, incluindo o sistema musculoesquelético. A ausência de um vetor de gravidade que estimule os músculos, leva a que se observe uma atrofia muscular por desuso durante uma missão espacial. Neste sentido, é de extrema importância e relevância compreender que medidas a podem atenuar sendo que intervenções ao nível da nutrição e do exercício físico têm demonstrado ser duas dessas medidas.

OBJETIVO

Esta revisão sistemática tem como objetivo adquirir e resumir o conhecimento atual sobre os impactos da combinação do exercício físico com a administração de suplementação nutricional na perda de massa muscular esquelética em estudos realizados com indivíduos saudáveis submetidos a períodos de alectuamento prolongado, "*bed rest studies*".

MÉTODOS

Foi realizada uma pesquisa abrangente da literatura em várias plataformas de dados científicos, seguindo as recomendações das diretrizes da PRISMA - 2020. Os resultados da pesquisa foram posteriormente inseridos na plataforma Rayyan, seguido pela remoção dos artigos duplicados. Dois revisores selecionaram todos os artigos elegíveis de forma independente, sem conhecer as escolhas do outro revisor. Os critérios de inclusão e exclusão estavam acessíveis a todos os revisores e colaboradores. Os artigos foram inicialmente triados com base no título e no resumo, e a revisão do texto completo foi realizada em caso de ausência de informações suficientes no respetivo título ou resumo para tomar uma decisão assertiva. A avaliação do risco de viés também foi realizada, seguida pela síntese e análise dos dados.

RESULTADOS

Foram identificados 266 artigos, dos quais cinco foram elegíveis para revisão sistemática. Os grupos que realizaram simultaneamente treino de resistência com ou sem vibração e suplementação com aminoácidos essenciais ou proteína *whey*, apresentaram uma menor perda de massa muscular durante o período de alectuamento quando comparados com grupos que apenas tomaram suplementação dietética ou apenas realizaram exercícios de resistência com ou sem vibração. Esta diferença foi ainda maior em relação a grupos que não foram submetidos a nenhuma dessas intervenções.

DISCUSSÃO

Conhecer as medidas mais eficazes para minimizar a perda de massa muscular causada pela permanência em ambientes de microgravidade permite um melhor planeamento e ação na prevenção da perda de massa muscular dos astronautas durante a missão espacial, bem como na recuperação pós-missão, garantindo, consequentemente, um desempenho e performance humana mais elevadas e eficientes. Adicionalmente, a aplicação destas contramedidas, pode possibilitar uma preparação física mais adequada da tripulação para enfrentar períodos de gravidade lunar/marciana em futuras missões. Por outro lado, é de interesse salientar que este conhecimento também pode ser amplamente aplicado em diversas áreas médicas.

Palavras- chave: *BedRest*; *Head-Down Tilt*; *Space Flight*; Microgravidade; Fraqueza muscular; Atrofia muscular; Exercício físico de resistência; Exercício físico com vibração; suplementação com aminoácidos essenciais; Proteína *Whey*

ABSTRACT

INTRODUCTION

The loss of skeletal muscle mass during space flight is a major challenge for Medicine in space exploration. Microgravity, encountered in the space environment, is responsible for the disruption of various physiological systems, including the musculoskeletal system. The absence a gravity that stimulates the muscles, leads to muscle atrophy due to disuse during a space mission. In this regard, it is extremely important and relevant to understand which measures can mitigate this muscle mass loss, with interventions in nutrition and physical exercise having shown to be two such measures.

OBJECTIVE

This systematic review summarizes the current understanding on the combined impacts of exercise therapy and dietary supplements on the loss of skeletal muscle mass in healthy adults submitted to prolonged bed rest.

METHODS

A comprehensive literature search was performed on scientific databases, following the recommendations of the PRISMA - 2020 guidelines. Search results were collected and uploaded to Rayyan, followed by removal of duplicate articles. Two reviewers subsequently selected all eligible articles independently without knowing the other reviewers' choices. Inclusion and exclusion criteria were accessible to all reviewers and collaborators. Titles and abstracts were firstly screened, and full-text review was performed in case of absence of enough information in the respective title or abstract to make an assertive decision. Risk of bias assessment was also performed, followed by data synthesis and analysis.

RESULTS

266 papers were identified, five of which were eligible for systematic review. In overall, groups that performed resistance or vibration training combined with essential amino acid or whey protein supplementation had a greater attenuation of muscle mass loss seen with disuse during bed rest, compared to groups that only took dietary supplementation or performed resistance or vibration exercise alone and even greater compared with groups that did not undergo any of these interventions at all.

DISCUSSION

Knowing the most effective countermeasures to attenuate the loss of muscle mass caused by weightlessness allows for better planning of in-mission muscle waste prevention and post-mission recovery of astronauts and, consequently, ensures higher human performance. Therefore, this may allow the possibility of having a better physically prepared crew to face lunar/mars gravity periods in future missions. This knowledge can, additionally, be broadly applicable in many medical areas.

KEYWORDS

BedRest; Head-Down Tilt; Space Flight; Weightlessness; Skeletal Muscle; Muscle Weakness; Muscle Atrophy; Resistance training; Vibration training; Essential amino acid supplement; Whey protein

LIST OF ABBREVIATIONS

ACSA – Anatomical cross- sectional area

BR – Bed Rest

EAA – Essential amino acids

HDT - Head-Down Tilt

ISS - International Space Station

PRISMA - Preferred Reporting Items for Systematic Reviews and Meta-Analyses

RCTs - Randomized Controlled Trials

RT – Resistance training

RVE – Resistive vibration exercise

SP – Supine position

WBV – Whole body vibration

WHO - World Health Organization

WP – *Whey* Protein

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I N T R O D U C T I O N

Space exploration is becoming more and more challenging as we move deeper and longer in space. This requires not only technological and engineering advances, but also new medical countermeasures to allow the minimization of all physiological adversities that the human body faces in this extreme environment.

Microgravity, defined as forces between ∼ 10−3 and 10−6 g[1], disrupts normal physiological systems, as demonstrated by medical examinations[2]. One of the most noticeable and well-described negative impacts is muscle wasting, or atrophy, caused by weightlessness – the disuse and unloading of muscles in microgravity.

Skeletal muscle atrophy is defined by a loss of muscle mass volume. Due to functional deficits brought on by this loss of muscle mass, there are clinical and practical repercussions[3]. Rather than a decrease in the number of muscle fibres, this atrophy is caused by a reduction in muscle fibre size, being the decrease in maximum force generation, the functional feature that is most obvious, despite not being proportional to the decrease in volume. Other functional deficits, however, include a rise in fatigability, a decline in speed, a rise in activation threshold, and a reduction in regenerative capacity[3]. It is, therefore, known that microgravity plays an important role in the loss of skeletal muscle mass in astronauts during spaceflight, especially prolonged spaceflight[4,5], resulting in significant deconditioning of the musculoskeletal system, mainly muscles with anti-gravity functions[6] such as the soleus muscles, the extensors of the leg, the gluteus maximus, the quadriceps femoris and the muscles of the back, mainly the multifidus. Some muscles from upper limbs such as arm adductors and elbow and wrist extensors and flexors are also included. Additionally, this can also be exacerbated by a negative energy balance. Studies also reveal that this deconditioning is believed to be potentially progressive, increasing with the extension of the duration of the mission[4,7].

Muscle atrophy is most noticeable in slow antigravity muscles and in muscles with a preponderance of type I fibres as opposed to type II fibres[3]. In microgravity studies where muscle biopsies were obtained during spaceflight, it is possible to observe a rapid transformation of Type I (slow) to Type II (fast) fibers[8,9]. Similar conclusions have been observed in human bed rest studies[10,11].

Bed rest studies, in which participants remain in bed for the duration of the experiment, are among the most notable examples of ground-based models used to simulate some of the effects of spaceflight[8].

Studies have also been showing that this spaceflight-induced muscle atrophy is similar to many terrestrial conditions[3,12]. One example is the loss of muscle mass observed in hospitalized patients due to longer periods of bed rest while in hospital. Loss of skeletal muscle mass influences the outcome and time of recovery of hospitalized patients, and it can be even seen during a short period of time, consequently leading to a longer period of recovery and a higher risk of disease recurrence[13]. Therefore, the study and understanding of what mechanisms are behind muscle wasting in space and how to attenuate it, also allows a clearer understanding of similar problems seen on Earth, opening a window to the discovery of more efficient ways to solve this problem. It is, therefore, of utmost importance and relevance to understand the mechanisms behind this deconditioning as well as the development of effective countermeasures.

Dietary interventions[13] and exercise therapies[14,15] have been shown to be countermeasures to attenuate the loss of skeletal muscle mass in long periods of muscle inactivity, such as bed rest.

During spaceflight, nutrition plays a variety of crucial roles, from sustaining metabolic requirements to even providing positive psychosocial benefits[3,16]. Undoubtedly, a key element in preventing the many negative effects of spaceflight, including muscle loss, is a proper, balanced diet. Muscle atrophy in space is more a result of reduced protein synthesis than it is of increased protein catabolism[3], being low calorie intake, which is frequently seen during extended space missions, a significant contributor to muscle atrophy. In fact, Smith et al.[17] observed that astronauts only consumed a mean of 80% of the necessary energy intake recommended by the World Health Organization (WHO) during a space shuttle mission.

Exercise, in turn, is crucial in the prevention of loss of muscle mass during spaceflight[5], being the average daily allotment of exercise time for astronauts aboard the International Space Station (ISS) 2.5 hours[18]. On earth, there is ample proof that resistance training is a successful strategy for halting muscle wasting and maintaining muscle function in a range of populations[19]. However, a crucial qualification is that, in order to maximise the chance of a synergistic anabolic response, resistance training should be combined with an adequate diet, high in protein and energy[19].

O B J E C T I V E

This systematic review pretends to synthesise the available data from existing randomized controlled trials to assess whether the combination of both exercise therapy and dietary supplements can attenuate the loss of skeletal muscle mass in healthy adults submitted to a period of bed rest.

M E T H O D S

This systematic review was structured according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 checklist[20].

ELIGIBILITY CRITERIA

Participants eligible (P) consisted of healthy adults, both genders, aged between 18 and 65 years old. Adolescents (under 18 years of age), elderly (over 65), adults with contraindications to exercise, dietary restrictions that prevent consumption of the dietary supplement(s) given, neuromuscular pathologies, musculoskeletal impairments, or presenting any other psychological or organic disease(s) were excluded.

Interventions eligible (I) consisted of exercise therapy (resistance training or vibration training) combined with a dietary supplement (essential amino acid supplementation or whey protein supplementation) during bed rest period (supine position or -6 degree head- down tilt).

Comparisons eligible (C) consisted of studies that compared subjects that did not undergo any resistance training, with or without vibration platforms, or/and did not take essential amino acid supplementation or whey protein supplementation during the bed rest period with subjects that performed resistance or vibration training and took either essential amino acid supplementation or whey protein supplementation.

Outcome Measures Eligible (O) consisted of studies that measured parameters that allowed the evaluation of the muscular atrophy and functionality of skeletal muscle.

Randomized controlled trials were included. Studies were excluded if they met the following criteria: (1) Studies other than randomized controlled trials (RCT), (2) Non- english articles, (3) Articles published before 2003, (4) Animal trials, (5) Subjects were excluded if they had any contraindications to exercise, dietary restrictions that prevent consumption of the dietary supplement(s) given, musculoskeletal impairments, tumours or were critically ill, or had any other psychological or organic disease(s), (6) the intervention group(s) did not include physical exercise combined with dietary supplementation, (7) Outcomes did not include outcome indicators of any interest.

SEARCH STRATEGY

A comprehensive literature search was performed on EMBASE, MEDLINE and Web of Science databases, following the recommendations of PRISMA - 2020 guidelines[20]. Search in "All

Fields” was performed on every platform, except for EMBASE, where a “Title, Abstract & Keywords” only rule was employed. The search was conducted on the 16th of October 2023.

The search key used was “(("bed rest") OR ("head down tilt") OR (spaceflight) OR (spaceflights) OR (microgravity) OR ("simulated spaceflight")) AND (("muscle atrophy") OR ("muscle weakness") OR ("muscle disuse") OR ("disuse atrophy") OR ("disuse muscular atrophy") OR ("muscle disuse atrophy") OR ("muscle mass") OR ("muscle strength") OR ("body composition") OR ("myogenic factors")) AND (("dietary supplement") OR ("dietary supplementation") OR ("dietary supplements") OR ("dietary supplementations") OR ("diet therapy") OR ("nutrition therapy") OR (supplementation) OR ("essential amino acids") OR (nutrition) OR ("amino acid supplementation")) AND (("exercise therapy") OR ("exercise therapies") OR ("resistance exercise") OR ("resistance training") OR ("vibration resistance training") OR (exercise))”.

STUDY SELECTION AND DATA COLLECTION PROCESS

Search results were collected and uploaded to Rayyan, followed by removal of duplicate articles. Two reviewers subsequently selected all eligible articles independently without knowing the other reviewers’ choices. Inclusion and exclusion criteria were accessible to all reviewers and collaborators. Titles and abstracts were firstly screened, and full-text review was performed in case of absence of enough information in the respective title or abstract to make an assertive decision. Any disagreements regarding inclusion of a certain article were discussed and resolved by a third reviewer. This process, therefore, followed PRISMA 2020 guidelines on study screening and selection[20].

DATA ITEMS

From the final selected studies, relevant data was extracted: (1) name of first author; (2) study characteristics (study design, setting, sample size); (3) year of publication; (4) study population; (5) number of participants in intervention and control groups; (6) age, sex and body mass index of participants; (7) type, dosage and duration of dietary supplements; (8) type, description, duration and weekly frequency of physical exercise; (9) type (supine position or head-down tilt) and duration of bed rest; (10) multiple parameters that allowed the evaluation of muscular loss and functionality of skeletal muscle.

STUDY RISK OF BIAS ASSESSMENT

Risk of bias assessment of the included studies was conducted using the revised Cochrane risk of bias tool for randomized trials (RoB2)[21] for the following domains: randomization process, deviations from the intended interventions, missing outcome data, measurement of the outcome and selection of the reported result.

Each parameter and the overall study were then evaluated in a scale that is comprised of "Low", "Some concerns" or "High" risk-of-bias levels, as well as "No information" available for risk of bias assessment[22].

DATA SYNTHESIS AND ANALYSIS

The initial intent was to perform a comprehensive statistical analysis to quantify and compare the effects of interventions on specific outcomes related to skeletal muscle mass and functionality. However, due to the heterogeneity in outcome measures employed by the included studies, a meta-analysis or comparable statistical synthesis could not be conducted. Given these limitations, the synthesis of results is presented in a narrative format, emphasizing qualitative patterns and trends observed across the studies.

R E S U L T S

STUDY SELECTION

Using the search strategy described, 409 articles were found, of which 266 remained after removing duplicated literature (143). 261 articles were further excluded after screening the respective titles and abstracts as they were not eligible either because they were not randomized controlled trials (RCTs), they were animal trials, or the intervention group(s) did not include physical exercise combined with dietary supplements. 5 final articles were included[23–27]. A flow chart of study selection is shown in **Figure 1**.

STUDY CHARACTERISTICS

Study characteristics of the 5 included RCTs are summarized in **Table I**. 93 subjects were enrolled in total, all of them were men, aged between 18 and 65 years old. The number of subjects per study ranged from 8 to 31. The dietary supplements given were whey protein in 3 studies[23–25] and essential amino acids in the other 2 studies[26,27]. The same 3 studies[23–25] had resistance vibration exercise in their protocol, whereas the other 2 studies[26,27] included only resistance exercises (with no vibration). Details of the dose of dietary supplementation and duration and frequency of exercise performed in each study as well as multiple parameters measured to infer muscle mass loss and functionality, are shown in **Table I**.

In all studies, participants were submitted to 3 phases: a phase of acclimatization and baseline data collection for 7[24–27] or 14 days[23] followed by a period of 28 days of bed rest (BR) in supine position[26,27] or 21 days of – 6 degree HDT[23–25] BR. The last phase was a post- BR active recovery period of 7[23–25] or 14 days[26,27]. 2[26,27] studies also compared if the moment of dietary supplementation, either before or after resistance training, had an influence on muscle mass loss and functionality.

RISK OF BIAS IN STUDIES

Risk of bias in the 5 included studies is shown in **Figure 2**. All studies included in the analysis received a consistent rating of "some concerns" for the overall risk of bias, mainly because of their assessment in the domain of "Deviations from the intended interventions'' which included the questions: (1) Were participants aware of their assigned intervention during the trial? - Answer: Yes (for all studies) (2) Were carers and people delivering the interventions aware of participants' assigned intervention during the trial? - Answer: Probably Yes (for all studies) (3) If

Yes/Probably Yes/No Information to 2.1 or 2.2: Were there deviations from the intended intervention that arose because of the trial context? - Answer: No information (for all studies).

This collective rating can be attributed to the inherent challenge of blinding subjects to the interventions during the testing period. The need for subjects' active cooperation in executing exercise protocols naturally exposed them to the nature of the interventions. It is important to mention that subjects were not informed about their specific group allocation in the start of the studies and throughout the initial testing phase preceding the bed rest period; the allocation process was randomized.

It is crucial to underscore that the compromise in blinding during the intervention period was primarily due to the practical necessity of subjects' cooperation, a key requirement for effective implementation of the study protocols (exercise and dietary protocols). Despite this challenge, the initial allocation remained concealed from participants during the crucial phases of study initiation and randomization, preserving the integrity of the study design.

Therefore, despite being rated as "some concerns" for the reasons mentioned above, it is crucial to emphasize that the studies included in the analysis remained valuable and eligible for consideration, affirming their relevance for informing future research and practice in the field.

SUMMARY OF RESULTS

It is possible to notice a very high diversity of outcome measurements that allowed the evaluation of muscle mass loss and functionality among the 5 final articles, which created some barriers for performing statistical analysis, as mentioned previously.

One article in particular, Patrick et al.[23], focused on the impact of adding whey protein supplementation to resistive vibration exercise (RVE) on lumbar paraspinal muscles during 21 days of -6 degree HDT-BR. Patrick et al.[23] compared 3 groups: one control group (CNT group) with no exercise protocol nor dietary supplementation, one with only RVE (RVE group) and one with both RVE plus whey protein (WP) supplementation (NeX group) and it was possible to see lumbar paraspinal muscles atrophy in the CNT and RVE groups, whereas in the NeX group, no significant muscle mass loss was found. This suggests that protein supplementation in combination with resistive vibration exercise can have better results than no interventions at all or exercise alone. Regarding the recovery period, it was seen that lumbar paraspinal muscle atrophy was still present on day 6 post-BR (days), but recovered after 28 days of post-BR. One important aspect to mention as well is that there was no specific rehabilitation done for this trial. Therefore, these results suggest that paraspinal muscles can recover on their own following a BR period without requiring special assistance[23].

Kenny et al.[24] and Liphardt et al.[25] also studied subjects submitted to a -6 degree HDT-BR, but with a focus on lower limb muscles. Both studies had a 21-day BR period with 3 groups similar to the ones done by Patrick et al.[23]: CNT group, RVE group and NeX group. The dose of WP given to the NeX group was the same (1.8 g/kg body wt/day) in both studies. The main difference between these two studies, apart from the outcome measurements, was the amount of RVE done per week: 2 days/week, 2-4 min/session time on Kenny et al.[24] study and 2 days/week, 25 min/session time on Liphardt et al[25]. Both studies were performed on an integrated training device (Novotec Medical GmbH) that combined a standard leg press machine with a vibration platform located on the foot plate and the same vibration parameters were used (8mm peak-to-peak, 25 Hz). Bilateral and unilateral heel raises (x1.8 and x1.3 body weight, contractions completed as quickly as feasible until fatigue) and bilateral squats (10 repetitions, 8s per repetition) were performed by subjects on Kenny et al.[24]

Kenny et al.[24] studied the physiological and proteomic responses of lower body RVE alone or with whey protein supplementation (NeX) and concluded that loss of leg strength could not be prevented by the RVE and NEX trials. However, numerous alterations in skeletal muscle proteomics, obtained with muscle biopsies, (muscle proteomics, TCP1, CAPN 1, 2 and 3 levels) were stopped by the RVE and NeX trials, which also showed a partially protective effect at the protein level. Additionally, Kenny et al.[24] could observe an increase in leg lean mass in the RVE group and an even more significant one in the NeX group. Proteomic data suggests that RVE, when paired with WP supplementation, may enhance protein synthesis during bed rest and this improvement is evidently more pronounced than RVE alone.

Liphardt et al.[25], in turn, aimed to determine whether RVE, alone or combined with WP, could attenuate muscle mass loss by measuring the anatomical cross-sectional area (ACSA) with magnetic resonance imaging in various muscles of the lower limb (rectus femoris, vastus lateralis, vastus intermedius, vastus medialis, quadriceps femoris, hip adductors, knee flexors and sartorius). It was possible to observe that, during the BR period, the ACSA of every muscle group, aside from the vastus medialis, decreased significantly within the 3 groups, including the NeX intervention. In other words, neither the RVE nor the NeX trials were able to stop the reduction of ACSA. On another perspective, more muscles recovered to baseline values in the NeX group during the 7-day post-BR recovery period. This finding may potentially suggest that the response to the RVE and NeX may not be evident until after several weeks of bed rest, as the study advanced into the recuperation period. This can be an aspect to consider when performing more studies similar to these ones.

Brooks et al.[26,27] studied subjects submitted to a 28-day BR period, but in supine position (SP), with essential amino acid supplementation (EAA) given and with resistance training (RT) instead of HDT, WP supplementation and RVE, respectively. 3 groups were also compared: one received only EAA supplementation (AA group), other RT with EAA given 3h after training (RT group) and the last one RT with EAA given 5 min before training (AART group). Both studies had the same exercise protocol (1h/day, 6 days/week, alternating days of lower body - squats, single leg squats, diagonal jump, calf raise, single-leg hip extension, leg curl, and single-leg hip abduction- and upper body - pull-ups, pull-over, triceps press, chest fly, shoulder press, biceps curl, upright row, and lateral arm raise - exercises, exercise intensity 70-80% of one-repetition maximum), energy intake calculation and nutrition plan (diet consisting of 15% energy from protein, 54% from carbohydrates, and 33% from fat; energy expenditure estimated from resting metabolic rate; total energy intake reduced by 8% during BR and recovery phase; 15 g of EAA supplementation given once per day, on the same 6 days of the week that RT and AART groups exercised, during BR and recovery), as well as same protocol for active recovery (3x/week, the 3 groups engaged in 15–30 minute exercise on treadmill at an intensity ranging from 60 to 85% of their age-predicted maximum heart rate; both duration and intensity were progressively increased over the course of 14 days).

From one of these studies[27], it was possible to observe greater loss of lean muscle mass of lower limbs, decrease in midthigh muscle area and lower body strength in the AA group compared with RT and AART groups. The effects of BR on muscle mass and muscle strength were nearly two-thirds lessened by RT and AART interventions. Hence, no significant differences were observed between these 2 groups, which might suggest that the losses of lean muscle mass and muscle strength were similarly mitigated during BR when resistance exercise training and an essential amino acid supplement were given either 5 minutes prior to activity or 3 hours after exercise. Therefore, the timing of EAA administration did not seem to be relevant, at least given this period (28 days) in BR.

In the other study, Brooks et al.[26] measured transcripts encoding atrophic, growth, and myogenic regulatory factors, as well as analysed satellite cells and myonuclei. It was possible to observe that RT and AART groups countermeasures stopped transcripts encoding myostatin, a protein that negatively regulates muscle hypertrophy by preventing the development, differentiation, and synthesis of new muscle fibres, from building up, preventing their accumulation. In other words, by the end of BR period, participants who received EAA alone had more myostatin transcripts in their muscles and, concomitantly, greater muscle atrophy, than those who were supplemented with EEA and performed resistance training, with no differences between RT and AART protocols. Hence, RT and AART groups maintained myonuclear levels following BR period, whether AA group showed a gradual decrease in myonuclei over time, which suggests a synergic effect when using both resistance exercise and EAA supplementation. These two articles, therefore, showed that the combination of resistance exercise with EAA supplementation had better effects on attenuating muscle atrophy when compared to EAA supplementation alone.

D I S C U S S I O N

Statistical Analysis Limitations:

One of the challenges encountered was the heterogeneity of outcomes measured across the selected articles. The initial intent was to perform a comprehensive statistical analysis to quantify and compare the effects of interventions on specific outcomes related to skeletal muscle mass and functionality. However, due to the variability in outcome measures observed in the included studies, a meta-analysis or comparable statistical synthesis was unfeasible and could not be conducted.

It is possible to say that this wide range of reported outcomes reflects the multifaceted nature of the interventions studied and the lack of standardized measurement protocols within the field. Given these limitations, and while a quantitative synthesis would have provided a more robust analysis, the synthesis of results was presented in a narrative format, emphasizing qualitative patterns and trends observed across the studies.

It is important to acknowledge this limitation in the review process to maintain transparency and guide future research directions in terms of standardizing outcome measures within the field and despite the inability to conduct a quantitative synthesis, the qualitative synthesis ended up by providing valuable insights into the overall trends and implications of the interventions on skeletal muscle outcomes.

Other limitations and further discussion:

It is crucial to bring up and discuss additional restrictions in addition to the ones that have already been covered. First off, one inherent constraint is that all studies that were analysed only included male participants. Although it may be tempting to extrapolate results to female participants, a specific and focused examination is necessary to understand the physiological and proteomic responses in females. Emphasizing the importance of carrying out additional studies aimed at the female population becomes imperative to guarantee a better and more complete comprehension of the relationship between physical activity, nutritional supplements, and skeletal muscle results in both genders.

The sample size of the studies considered can also be a noteworthy drawback, as there may be some concerns regarding the robustness and generalizability of the results due to the small sample sizes. This can, consequently, restrict the data's wider applicability and make it more challenging to reach statistical significance. It would be interesting to have larger and more varied participant cohorts, as it could improve the external validity and reliability of the observed results in future research in this area.

One other aspect regarding the study populations must be noted, and it is present in all of the examined studies: all the studied populations consisted of exceptionally healthy subjects with no prior medical conditions. This characteristic limits, therefore, the findings' applicability to larger populations with a range of health issues. Because skeletal muscle responses to bed rest, exercise, and dietary supplementation can differ significantly among individuals with health issues, the small sample size of the study and type of selected population can raise concerns when it comes to extrapolating these findings to people with underlying health issues or diverse physiological profiles. This becomes much more important when one considers the implications on space exploration, where future astronauts may possess a range of health conditions. The extrapolation of findings from this review to such a population warrants careful consideration, emphasizing the need for future research to include individuals with diverse health statuses, ensuring that the insights gained are applicable to the anticipated pool of space travellers.

Moreover, thoughtful consideration should also be given to the duration of the bed rest period in each of the included studies. Although the selected time intervals allowed an investigation of rapid responses, it is relevant to question whether they were adequate to record all morphological and physiological changes generated by study protocols. Extended bed rest may produce distinct responses over time. Therefore, it would be interesting to have future studies with different bed rest durations to clarify the temporal dimensions of skeletal muscle adaptations and address any potential drawbacks related to the chosen durations in the reviewed studies.

Regarding the exercise protocols, resistance exercise was performed in lower limbs in Patrick et al.[23], Kenny et al. [24] and Liphardt et al.[25] studies, while Brooks et al.[26,27] applied upper and lower limb exercises in their exercise protocols. Following this line of thought, it could be interesting to have future studies consider whole body resistance exercise, with or without whole body vibration (WBV) and evaluate the impact of these countermeasures on other muscle groups. Three[23–25] of the five studies also included WBV. Although in spaceflight analogue research, WBV alone does not appear to play a significant role in maintaining muscle volume after a period of BR[28], or the fact that some studies suggest that there might be no clear evidence of its effects on size, strength, and contractile speed in lower limb muscles[29], WBV has been looked into as a way to increase the effectiveness of resistance exercise, having an additive benefit[30–32]. By standing on a vibrating platform and consequently exposing the body to a vibration stimulus while performing resistance training, WBV has been suggested as a promising countermeasure by having the ability to not only preserve muscular strength, but also prevent bone loss[33,34].

On the other hand, vibration itself is a very important aspect to consider during spaceflight. Vibrations from crew activities and equipment operations can negatively impact several studies carried out in Earth-orbiting laboratories. Therefore, vibrations connected to the use of certain exercise equipment, needed to maintain physical strength and conditioning, may raise some concerns[35]. Even though aerobic exercises, such as bicycle ergometer and treadmill exercises, have isolation systems and resistance exercise devices do not usually cause vibrations[35], adding vibrating platforms to allow crew members to have WBV while performing on these resistance exercise devices should be assessed to see whether it can potentially increase overall vibrations and interfere with some scientific experiments. Following this line of thought, WBV could still be a very crucial countermeasure to be added to resistance exercise in contexts such as post-mission recovery instead of during-mission prevention or implemented when on Lunar or Martian surface in future missions.

Still regarding the topic of exercise, it is noteworthy to highlight a gap in the available data across the studies examined: the absence of comprehensive information regarding the exercise history and fitness levels of the participants. The reviewed studies did not provide details on whether participants had previously engaged in regular exercise or not (baseline of daily physical activity), the frequency and intensity of such exercise, or their overall fitness status. This lack of information introduces a significant variable that could potentially influence the final outcomes observed, as it is hard to rule out the possibility that individuals' previous exercise routines, or lack of them, have not had an impact on how they react to bed rest, exercise protocols, and dietary supplements. Understanding the baseline fitness and exercise status of participants is crucial for contextualizing and interpreting the observed effects of interventions. Therefore, it would be interesting to observe in future research studies the inclusion of detailed information about participants' exercise backgrounds to enhance the validity and applicability of findings in the broader context of skeletal muscle responses to bed rest interventions.

Looking at nutrition itself, Brooks et al.[26,27] and Patrick et al.[23] opted for hypocaloric diets, while Liphardt et al.[25] chose a normocaloric diet. The decision between a hypocaloric and a normocaloric diet involves a complex interaction of physiological and metabolic factors in the context of bed rest experiments that simulate microgravity. An energy deficit may be induced by a hypocaloric diet, which is characterized by a lower calorie intake. This deficiency may accelerate the loss of skeletal muscle when combined with the deconditioning effects of simulated microgravity on the muscles. The reduced availability of energy may intensify the catabolic processes[36], making it more difficult for the organism to sustain muscle mass when it is inactive for extended periods of time. On the other hand, a normocaloric diet, which provides sufficient calories to fulfill daily energy needs, attempts to reduce the risk of excessive muscle catabolism linked to bed rest-induced disuse by offering a balanced calorie intake. Understanding the complex effects of nutrition as well as the quality of the caloric intake on skeletal muscle responses to simulated microgravity becomes vital to maintain energy balance. An additional degree of complexity is added by the unique metabolic adaptations caused by a hypocaloric diet, such as modified substrate use and possible hormonal changes[37]. These adaptations may impact the overall metabolic and physiological response to bed rest, and they may differ from those seen under normocaloric conditions. Investigating these differences is crucial to understand the different impacts of dietary plans on skeletal muscle responses in a microgravity simulation environment.

Another important aspect to discuss is the position of subjects during bed rest: supine position[26,27] or -6 degree HDT[23–25]. Currently, the gold standard for simulating the effects of microgravity on the locomotor system and testing countermeasures for bone loss and muscular atrophy, orthostatic intolerance, and decreased muscle strength/exercise capacity is HDT bed rest[8,32]. On earth, 70% of the fluids of human body are below the level of the heart, while the other 30% are above it. In space, 50% of fluids are below this level, and the other 50% are above. Therefore, a -6 degree HDT induces a redistribution of bodily fluids, particularly towards the upper body, mimicking the fluid distribution changes that occur in microgravity environments, such as during space travel. A -6 degree angle is also practical and well-tolerated for extended bed rest periods, allowing participants to remain in this position for an extended duration without experiencing excessive discomfort or negative health effects. This is crucial for conducting controlled experiments where participants need to adhere to the bed rest protocol for an extended period. This, additionally, helps advance our understanding on how the human body responds to conditions similar to those experienced in space, giving valuable insights for space medicine and long-duration space missions.

Although it was possible to draw some conclusions from these articles, more studies are needed to allow a deeper understanding of the impact of resistance training (with and without body vibration) associated with protein or EAA supplementation on skeletal muscle mass and function during weightlessness and disuse, namely examine various combinations of these countermeasures and establish optimal protein doses and timing of supplementation as well as duration and frequency of a variety of exercise modalities and intensities. Following this line of thought, more research is also required to completely comprehend the intricate molecular regulators of muscle regeneration and atrophy in ground-based models of disuse, such as bed rest, and to determine the effects of dietary supplementation and resistance training on these pathways.

Understanding the most effective countermeasures to mitigate the loss of muscle mass induced by weightlessness is critical for planning both in-mission muscle preservation and post-mission recovery for astronauts. By implementing optimal interventions, space agencies can enhance the physical readiness of astronauts, ensuring high human performance during space missions. This knowledge not only equips space missions with effective strategies to minimize the impact of microgravity on muscle mass but also sets the stage for more robust recovery protocols upon astronauts' return to Earth.

Furthermore, the insights gained from these studies extend beyond the realm of space exploration. The exploration of interventions to counteract muscle wasting contributes to improving musculoskeletal health. Findings from space-related research can inform and advance medical practices on Earth, offering innovative strategies for preventing and managing muscle atrophy in various clinical scenarios. This interdisciplinary approach ensures that the knowledge acquired in the pursuit of space-related objectives becomes a valuable asset in addressing terrestrial healthcare challenges.

In anticipation of future lunar and Mars missions, where astronauts will face diverse gravitational environments, the importance of optimizing countermeasures becomes even more important. A comprehensive understanding of effective strategies for preserving muscle mass allows space agencies to prepare physically resilient crews. The translational impact of this knowledge, spanning from space exploration to terrestrial health applications, underscores the far-reaching implications of advancing our understanding of muscle physiology in altered gravitational conditions.

C O N C L U S I O N

By analysing the existing literature, it was possible to observe various approaches to address a common query: How does the combination of exercise and dietary supplementation influence skeletal muscle mass loss during bed rest? In fact, upon close examination of the five selected articles, it is possible to infer that the concurrent implementation of exercise and dietary supplementation can possibly better attenuate skeletal muscle mass loss compared to scenarios involving either exercise or protein supplementation alone, or the absence of both exercise and protein supplementation altogether.

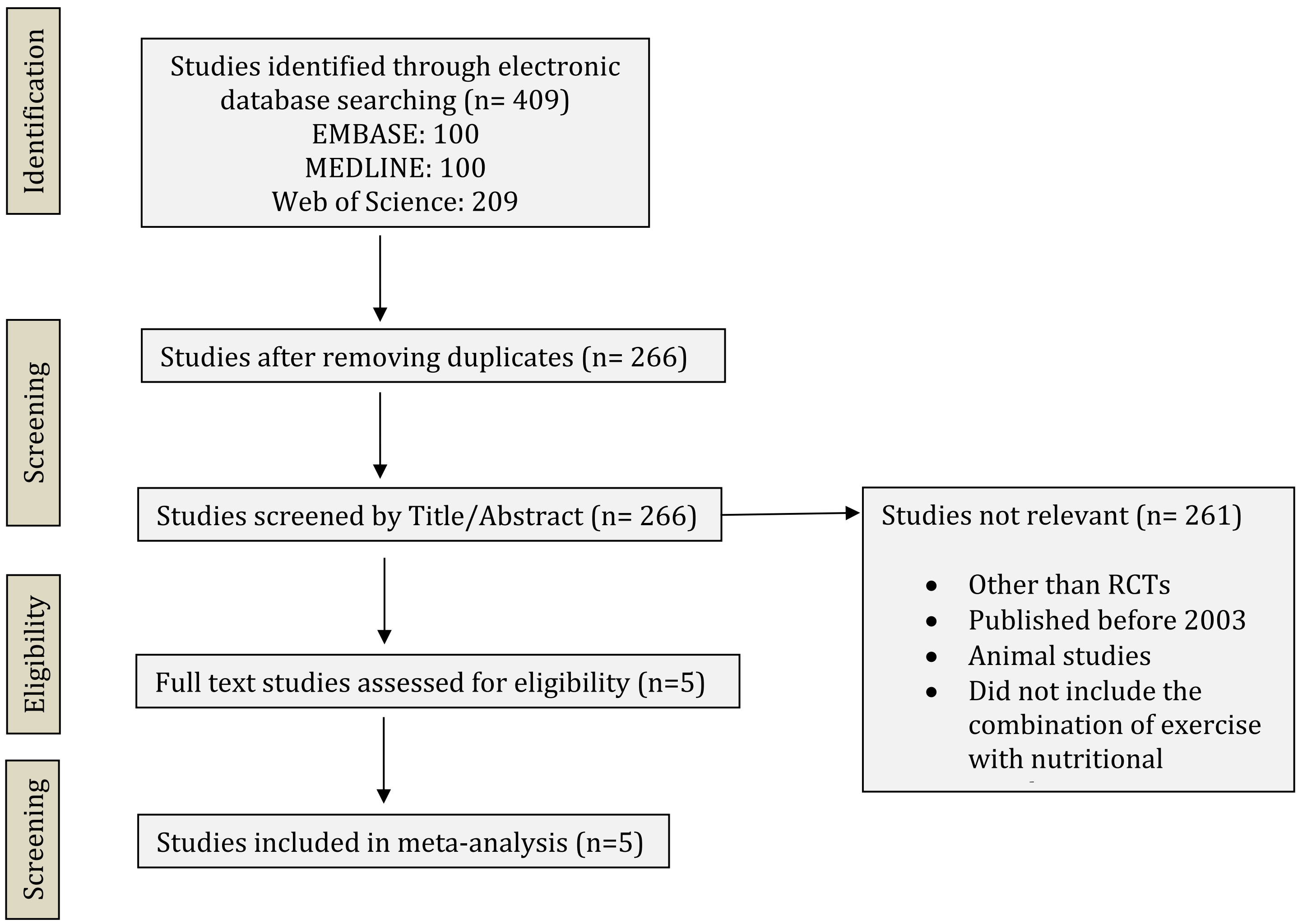
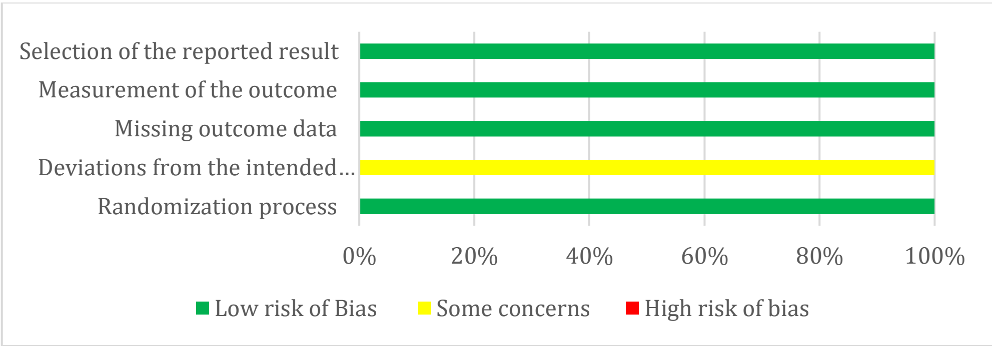


Figure 1 - Flow chart of study selection.

Table I - Characteristics of the studies included in the review. BMI, Body Mass Index; M/F, Male/Female; HDT, Head-Down Tilt; BR, Bed Rest; ACSA, anatomical cross-sectional area; EAA, Essential amino acid; WP, Whey Protein, RVE, Resistance Vibration Exercise; RT, Resistance Training

Study	Disuse Muscular Atrophy; Number of participants (P)	Control Group			Intervention Group			Outcomes
		Type	Dose, duration	Population (age, number, sex, BMI)	Type	Dose, duration	Population (age, number sex, BMI)	
Patrick X et al. [23]	21 days of - 6-degree HDT BR 12 P	No RVE + no WP	0 exercise + 1.2 g/kg body wt/day protein	Age (y): 35.2 (8.1) Sex (M/F): 11/0 BMI: No data	RVE + WP	2 days/week, 2-4 min/session time under tension + 1.8 g/kg body wt/day WP	Age (y): 36.2 (6.9) Sex (M/F): 9/0 BMI: No data	Atrophy of the lumbar (L1–L5) paraspinal muscles
Kenny H et al.[24]	21 days of - 6-degree HDT BR 8 P	No RVE + no WP	0 exercise + 1.2 g/kg body wt/day protein	Age (y): 35.18 (8.1) Sex (M/F): 8/0 BMI: 22.57 (1.7)	RVE + WP	2 days/week, 2-4 min/session time under tension + 1.8 g/kg body wt/day WP	Age (y): 35.18 (8.1) Sex (M/F): 8/0 BMI: 22.57 (1.7)	Leg lean mass, maximal isometric voluntary contraction; muscle proteomics, TCP1, CAPN 1,2 and 3 levels
Liphardt AM et al. [25]	21 days of - 6-degree HDT BR 12 P	No RVE + no WP	0 exercise + 1.2 g/kg body wt/day protein	Age (y): 35.2 (8.1) Sex (M/F): 11/0 BMI: 22.6 (1.7)	RVE + WP	2 days/week, 25 min/session time + 1.8 g/kg body wt/day WP	Age (y): 37.1 (6.7) Sex (M/F): 8/0 BMI: 23.0 (1.78)	ACSA of lower limb
Brooks NE et al. [26]	28 days of supine position BR 31 P	No RT + EAA	0 exercise + 15g EAA (6x/week)	Age (y): 31–55 years) Sex (M/F): 7/0 BMI: 23–31 kg/m2	RT + EAA (5 min before or 3h after RT)	6 days/week, 60 min/session time + 15g EAA (6x/week)	Age (y): 31–55 Sex (M/F): 12/0 // 12/0 BMI: 23–31 kg/m2	myostatin transcript levels, Pax7, MyoD, myogenin, MuRF1 and MRF4 transcripts, MAFbx transcripts

Brooks N et al.[27]	28 days of supine position BR
	
	31 P
		No RT + EAA	0 exercise + 15g EAA (6x/week)	Age (y): 44 (9)
				Sex (M/F): 7/0
				BMI: 25.6 (3.5)
					RT + EAA (5 min before RT // 3h after RT)	6 days/week, 60 min/session time + 15g EAA (6x/week)	Age (y): 42 (8) // 44 (6)
							Sex (M/F): 12/0 // 12/0
							BMI: 26.3 (2.7) // 25.5 (2.3)
								Lean body mass, Leg lean mass, Arm lean mass, Midthigh muscle area, Midthigh intramuscular fat area, Lower body muscle strength, Upper body muscle strength



A

Patrick X et al. [23]	Kenny H et al.[24]	Liphardt AM et al. [25]	Brooks NE et al. [26]	Brooks N et al.[27]	B
					Randomization process
					Deviations from the intended interventions
					Missing outcome data
					Measurement of the outcome
					Selection of the reported result
					Overall risk of bias

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

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