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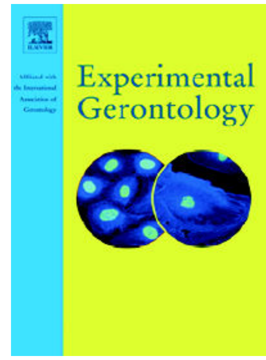

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No impact of combining multi-ingredient supplementation with exercise on body composition and physical performance, in healthy middle-aged and older adults: A systematic review and meta-analysis

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Abstract

Background: Protein-based multi-ingredient (MTN) supplements have been suggested as a safe and effective way of enhancing exercise outcomes. However, their effectiveness remains controversial when compared to isocaloric and single-nutrient supplements. This review aims to systematically summarise the current knowledge of multi-ingredient supplementation to optimise body composition and physical performance in middle-aged and older adults.

Material and Methods: A systematic review with meta-analysis was performed according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA). The search of the literature was conducted using PubMed, EBSCO*host*, Google Scholar, Web of Science, and SPORTDiscus from June to October 2021. Every publication identified from the outset to October 2021 was considered. The main inclusion criteria comprised randomised controlled trial (RCT) studies conducted in adults (≥45 years old), following resistance- or endurance-based training programmes for a period of 6 weeks or longer, combined with MTN supplementation and a calorie equivalent comparator (COMP) supplement (e.g., carbohydrates). Continuous data on body composition [fat-free mass (FFM) or lean body mass], strength, and functional capacity as markers of physical performance were pooled using a random-effects model.

Results: Initially, 3329 publications were identified. Data from nine RCTs were ultimately included, involving 476 participants. The overall quality of the included studies was high, demonstrating a low risk of bias. Compared to COMP, no significant further benefits of ingesting MTN were identified for FFM (kg) (g=0.044, 95% CI −0.14 to 0.22), upper-body strength (kg) (g=0.046, 95% CI −0.24 to 0.33), lower-body strength, leg press exercise (kg) (g=0.025, 95% CI −0.26 to 0.31), leg extension exercise (kg) (g=0.106, 95% CI −0.15 to 0.36) and functional capacity (time in seconds) (g=0.079, 95% CI -0.12 to 0.27).

Conclusions: No additional benefits of ingesting MTN vs. COMP to maximise exercise-induced outcomes on body composition and physical performance in healthy physically active middle-aged and older adults have been identified.

Keywords: multi-nutrient; supplement; lean mass, functional capacity, elderly

1. Introduction

An active lifestyle combined with appropriate nutritional patterns has proven to attenuate age-related impairment in quality of life by preserving muscular performance and improving functional capacity (von Berens et al., 2018). The term functional capacity has been used to describe a person's ability to perform domestic and self-care activities free of physically related limitations (Seitsamo, Tuomi, and Martikainen 2007). In this regard, resistance training (RT) combined with a daily protein intake higher than 0.8 g/kg of body mass (Thomas et al., 2016) has proven to be an effective strategy for attenuating age-related declines in functional capacity (Beasley et al., 2013; Seesen et al., 2020; Traylor et al., 2018), and improving musculoskeletal function in middle-aged and older adults (Bunout et al., 2004). Indeed, a well-structured RT programme successfully maintained or even increased muscle mass, muscular strength, and functional capacity in sedentary, obese, sarcopenic, active and trained, middle-aged and older adults (Clark, 2016; Fragala et al., 2019; Garber et al., 2011; Giallauria et al., 2015). The observed health-related benefits are maximised when RT-based interventions are combined with appropriate eating behaviours. For instance, adding whey protein-based admixtures, including carbohydrates, small amounts of fats and some essential (e.g., leucine) or conditional (e.g., glutamine) amino acids has been proven to maximise exercise outcomes in young adults (Candow et al., 2006; Davies et al., 2018; Naclerio and Larumbe-Zabala, 2016). However, conflicting results regarding the advantage of combining resistance exercise with protein-based multi-ingredients (MTN) supplements in healthy and pre-conditioned middle-aged and older adults have been published (Eliot et al., 2008; Sugihara Junior et al., 2018). MTN are specialised forms of supplements combining macronutrients, micronutrients, and other nutritional substances (amino acids derivatives, or stimulants) that may optimise exercise outcomes compared to exercise alone (Bell et al., 2017). Most MTN admixtures contain a proprietary blend of ingredients expected to promote benefits when taken as described. For instance, based on their specific formulation, MTN are recommended to be ingested prior, during, and or post-exercise, at breakfast or before sleep (O'Bryan et al., 2019). The ingestion of MTN before and during workouts has been recommended to enhance motivation to train and to maximise exercise performance (Damas et al., 2015; Puente-Fernández et al., 2020). On the other hand, ingesting MTN after exercise has been proposed to speed up recovery

(Naclerio et al., 2020, 2021) and eventually maximise training-induced outcomes (Bell et al., 2017), e.g., muscle mass gain, strength, and general functional capacity.

Older sedentary individuals experience anabolic resistance, which is characterised by a blunted muscle protein synthetic response to dietary amino acid ingestion at rest and after resistance exercise (Moore, 2021). Therefore, ingesting more protein per meal has been recommended to overcome anabolic resistance and maintain muscle mass in untrained adults (Wall et al., 2015). However, it is still unclear whether older trained persons need higher daily protein intake to benefit from exercise-induced muscle protein synthesis as observed in young individuals (Moore, 2021). Nonetheless, when the appropriate amount of daily protein (i.e., 1.6 g/kg) is consumed, it seems that adding protein-based supplements would not induce significant improvement in training outcomes in ~~both young and~~ old individuals (Morton et al., 2018).

Two previous systematic reviews conducted in adults observed beneficial effects of adding protein-based supplementation to optimise resistance training outcomes for middle-aged > 45 years old (O'Bryan et al. 2019) and ≥60 years old individuals (Liao et al. 2017). However, these reviews included studies using healthy, obese, and patient individuals that were meta-analysed together without considering the impact of health or body composition status on the adaptations induced by training and nutrition combined interventions (Hita-Contreras et al. 2018; Martínez-Amat et al. 2018). For instance, (Liao et al. 2017) included overweight and obese participants while (O'Bryan et al. 2019) included participants diagnosed with sarcopenia. Consequently, an analysis including only healthy individuals accounting for their particular response to combining exercise with MTN supplementation is still necessary. Therefore, the aim of the present systematic review and meta-analysis was to analyse the effects of MTN supplementation on training-induced improvements in body composition and physical performance (i.e., muscular strength, and functional capacity), on healthy and recreationally active middle-aged and older adults (>45 years old).

2. Methods

The analysis method and inclusion criteria were specified in advance and documented in a protocol registered at the International Prospective Register of Systematic Reviews, PROSPERO (CRD42020200336).

2.1. Search strategy

A systematic review of the literature was conducted in accordance with the recommended criteria provided in the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement (Liberati et al. 2009; Page et al. 2021; Shamseer et al. 2015) and the guidelines described for systematic reviews in the nutrition field (Moher and Tricco, 2008).

The procedures incorporated for the current meta-analysis included: identification, screening, eligibility, and inclusion/exclusion of studies. The search of the literature was systematically reviewed by using PubMed, EBSCO*host* (Medline, Academic Search Premier, CINAHL Plus with Full Text), Google Scholar, Web of Science, and SPORTDiscus through June to December 2021 (with no lower date limit).

We identified English and Spanish languages randomised controlled trials (RCT) conducted in human populations eligible for review, including articles, abstracts from annual scientific conferences and congress presentations, or doctoral theses as well as retrieved pre-printed versions via University Resources, without any assigned DOI. Commentaries, reviews, or duplicate publications from the same study were not included in this analysis. Additional studies were identified by contacting experts in the field as well as reviewing other systematic reviews previously published in similar populations and topics (Labata-Lezaun et al., 2020; Luo et al., 2017; Morton et al., 2018; O'Bryan et al., 2019). The reference lists of the retrieved studies were hand-searched to identify potentially eligible studies not captured by the electronic searches. Two reviewers (JPF and FN) independently screened the title, abstract and reference list of each study to locate potentially relevant studies. Any discrepancies between the two authors were resolved through consensus or by the opinion of a third author (ELZ).

Combinations of the following keywords were used as search terms: "((*Multi* OR Protein* OR Beef Protein OR Soy Protein OR Pea Protein OR Rice Protein OR Whey* OR BCAA* OR Branch*) AND (Supplement* OR Enrich* OR Formula* OR Combin* OR Fortifi*) AND ((Adult* OR Middle-Age* OR Old* OR Elderl* OR Ageing OR Aging OR Master Athlet* OR Senior Athlet*))) AND ((Resist* OR Endur* OR Aerobic Training OR Anaerobic Training OR Train* OR Exercise OR Strength* OR Power* OR Recov* OR Energ* OR Performance))) AND ((Body

Composition OR Body* OR Fat* OR Lean* OR Muscle OR Muscul* OR Skelet* OR Weight OR QoL OR Qual* OR Lif* OR Hypertroph* OR Metaboli* OR Capacity OR Function*))) NOT (=(Ill* OR Canc* OR Frail* OR Sick* OR ICU OR Sclerosis OR Diabet* OR Patient* OR Hospit* OR Rehab* OR Child* OR Kid* OR Toddler* OR Animal* OR Rat* OR Mice OR Mouse))".

2.2. Eligibility Criteria

The inclusion and exclusion criteria were the following: (i) the trial was randomised and controlled involving at least two groups: treatment and comparator (using an iso-energetic comparator (COMP) treatment including only one macronutrient or a placebo); (ii) the treatment combined prolonged (≥6 weeks) exercise-training intervention (resistance, endurance, flexibility, or mixing modalities) with a minimum workout frequency of 2 days per week while ingesting a MTN supplement containing protein from animal (e.g., whey, casein, beef, etc) or plant based sources (e.g., soy, rice, pea, etc) combined in a singular admixture with at least one more macro or micronutrient; (iii) the study measured primary outcome variables related to body composition such as lean body mass (LBM) or fat-free mass (FFM), upper and lower body strength estimated from the 1 repetition maximum test (1-RM), and markers of physical performance such as the 10-meters walking (10-MW), 6-minutes walking (6-MW), 5-times sit-to-stand (5-STS), 30-seconds sit-to-stand (30-STS), or timed up-and-go (TUG) tests; (iv) participants were 45 years old or older, free from health related disorders (e.g., acute or chronic illness, disease, or injury) and not taking any medication. Therefore, interventions in patients affected by obesity, diabetes mellitus, osteoporosis, cancer, HIV, or following a rehabilitation intervention after injury, etc, were not included. Additionally, as sarcopenia has been defined as a muscle disease (Cruz-Jentoft et al., 2019), studies conducted in sarcopenic populations were dismissed; (v) the effects of the treatment were compared to the effects of an iso-energetic comparator (COMP) treatment including only one macronutrient, e.g., only protein or carbohydrate; (vi) data on total calories provided by the multi-ingredient or COMP were available; (vii) dietary intake was monitored or eating pattern advises were provided; (viii) studies including remarkably lower (≥100 kcal) or no energy COMP were not included, as it has previously been demonstrated large differences in caloric intake appears to be one of the most relevant factors affecting training adaptations during middle- to long-term exercise interventions (McLellan et al., 2014). In addition, diet interventions and modifications, as well as interventions with food products, were not considered; (ix) the publication presented sufficient data to calculate the mean differences; and (x) abstract was published. These criteria support the notion that the only difference between the experimental (MTN) and COMP groups was attributed to the supplement intervention, and at least one of the aforementioned outcomes (LBM, FFM, upper or lower body strength, and/or functional capacity) was analysed. There were no restrictions on the number of participants, nor for sex or level of performance (e.g., minimum 1-RM). Studies that included participants with a recent history (less than 1 month before the intervention) of supplementation consuming protein, creatine, amino acids, or derivatives such as beta-hydroxymethylbutyrate (HMB) at baseline screening were excluded.

2.3. Study Records

2.3.1. Data Management and Selection

Potentially relevant articles were selected by (i) screening the titles; (ii) screening the abstracts; and (iii) if abstracts did not provide sufficient data, the entire article was retrieved and screened to determine whether it met the inclusion criteria; (iv) when data were not accurately presented (only available from figures or graphs), authors were contacted and asked to provide the appropriate range of values (included the raw data). When no answer was obtained but figures were available, values were estimated by using the PDF Adobe Acrobat Pro measuring application. Thereafter JPF and FN met to decide whether the selected articles matched and fitted the purpose of the review.

2.3.2. Data Collection Process and Coding

The following qualitative and quantitative information was extracted from each included study: (1) authors; (2) publication year; (3) baseline population characteristics; (4) intervention type including the exercise programme configuration [exercise mode (resistance or endurance) volume, intensity, and frequency] (5) control procedures; (6) study duration; (7) blinding; (8) sample size per group; (9) nutrient profile of the administered supplements and comparator treatments; (10) methods of ingestion and dose; (11) study compliance; (12) diet assessment; (13) outcomes measured at pre- and post-intervention; (14) group means and standard deviations (SD) for the following outcomes: (i) LBM or FFM; (ii) 1-RM values for upper body and lower body exercises (iii) scores obtained in the functional tests (e.g., 5 times sit-to-stand or timed-up-and-go). Regarding the effects of MTN supplementation on FFM or LBM, the definition of FFM excludes lipids in the cell membranes, central nervous system, and bone marrow, while LBM is an anatomical term that would include some or all of these (Wang et al., 1998). However, both variables share the muscle mass as the main component that would express changes as a consequence of exercise-related interventions in middle and older adults. Therefore, the outcomes affecting these variables have been analysed together. Regarding 1RM values, to reduce bias caused by different exercise modalities and assessments methods, only resistance exercises executed with free weight or weight machines, e.g., bench press, chest press, leg press, or leg extension were considered as valid outcomes to express changes in strength. Furthermore, isometric strength assessments were also included and analysed separately when appropriate.

2.4. Risk of bias in individual studies

Methodological information regarding the potential impact of bias was critically examined. Two reviewers (JPF and FN) ascertained individual study information independently as part of the quality control process. For each study, seven domains from the Cochrane collaboration tool for assessing the risk of bias (Higgins et al., 2019) were scored with high, low, or unclear risk for bias: sequence generation, allocation concealment, blinding of participants and personnel, blinding of outcome assessment, incomplete outcome data, selective outcome reporting and "other" issues (similarity in baseline characteristics and timing of outcome assessment). These seven domains assess the level of risk regarding selection bias, allocation bias, performance bias, detection bias, attrition bias, reporting bias and other biases. The aforementioned two researchers performed the quality assessment independently, and their findings were compared and discussed until consensus was achieved. Each domain was scored as −1 for high risk, 0 for unclear risk, and 1 for low risk. Scores were then summed with a possible range of scores from −7 to 7.

2.5. Data Analysis

Provided the selected studies demonstrated no significant heterogeneity ($p > 0.10$ and $I^2 < 50$), a meta-analysis was performed using the Comprehensive Meta-Analysis Software, version 2.2.064 (Biostat, Englewood, NJ). The random-effects model was selected based on the assumption of variability of true effects between studies. Four or more studies per outcome were required to generate weighted group mean differences, 95% confidence intervals (CIs), and corresponding p values for heterogeneity. From the collected data, we used the pre and post values of mean, standard deviation (SD), and sample size, for both MTN and COMP groups. Pre and post SD values were calculated when studies reported standard error instead of SD. The effect size was calculated using the Hedges' g. The primary meta-analysis compared the effects of MTN interventions combined with any type of training protocol, which was considered the experimental treatment (without any distinction between each MTN composition) vs. COMP in the analysed outcomes (LBM or FFM, upper and lower body strength, and functional capacity). When a quantitative analysis was not possible, a summary of the critical facts and results of the observed outcomes was considered. If heterogeneity and sensitivity analyses were considered significantly high, no data were meta-analysed and only individual results have been reported. Additionally, we examined the presence of studies with inflated standardised residual values (above 1.96 or below -1.96) to consider them as outliers. Publication bias was assessed using funnel plots of effect size (horizontal axis) by the standard error (vertical-axis), the "Trim and fill" procedure for the random effects, and the Orwin Fail-Safe N analysis.

3. Results

3.1. Study selection

Figure 1 describes the search strategy. The preliminary search identified 3329 relevant references. After examining all the retrieved titles, 806 publications were selected. Of those, 745 were excluded based on the abstract review. The remaining 61 publications were fully read and carefully examined by two reviewers. After this examination, 52 studies were excluded resulting in a total of 9 studies (Arnarson et al., 2013; Bell et al., 2017; Candow et al., 2006, 2008; Holwerda et al., 2018; Krause et al., 2019; Leenders et al., 2013; Nabuco et al., 2018, 2019) included in the meta-analysis.

Fig. 1

3.2. Characteristics of the Included Studies

The overall quality of the included studies was high, with a low risk of bias, scoring between 3 to 6 points in the Cochrane collaboration tool. A table showing the assessment for each study is provided as electronic supplementary material, Appendix S1.

Nine studies reported results from 19 valid MTN or COMP groups, including a total of 427 participants, 214 men and 196 women (all postmenopausal) from 59 to 91 years old, met the inclusion criteria (Figure 1). Publications from Nabuco et al. (Nabuco et al., 2018, 2019) were based on the same intervention and with the same participants, but two different manuscripts analysing different outcomes were published. Therefore, those participants were counted only once for the final calculation. The publication dates range from 2006 to 2019. The characteristics and main data of these studies are summarised in Table 1.

Table 1

Sample sizes ranged from 30 to 141 participants, with similar demographic characteristics across the studies. The MTN treatment included 9 to 75 participants while 10 to 66 subjects were assigned to the COMP condition. All selected RCT followed a parallel design. The studies by Bell et al. (2017), Candow et al (2006), and Nabuco et al. (2018, 2019) included recreationally active or untrained participants not involved in resistance exercise programs for the previous 6 months. Arnarson et al. (2013) and Holwerda et al. (2018) included participants not engaged in regular exercise programmes for 3 years before the beginning of the study, while Leenders et al. (2013) recruited participants with regular physical activity before 5 years of the beginning of the study. Krause et al. (2019) included only sedentary participants. All these studies combined a nutritional intervention with progressive resistance training programmes composed by multi-joint and global exercises as primary movements implemented over 10 (Candow et al., 2008), 12 (Arnarson et al., 2013; Candow et al., 2006; Holwerda et al., 2018; Krause et al., 2019; Nabuco et al., 2018, 2019) and 24 weeks (Leenders et al., 2013) (Table 1).

Eight studies (Arnarson et al., 2013; Bell et al., 2017; Candow et al., 2006, 2008; Holwerda et al., 2018; Leenders et al., 2013; Nabuco et al., 2018, 2019), involved resistance training programmes using free weights or machines performed 3 times per week on alternate days, while Krause et al. (2019) combined exercises performed with participants' body mass and elastic bands. Overall, all the included studies used moderate (~60% to 70% of the estimated 1-RM) to heavy (~80% of the estimated 1-RM) overloads, 3 to 4 sets of 15, 10, 8 or 6 repetitions per exercise, and ~2 min rest between sets.

All nine studies (Arnarson et al., 2013; Bell et al., 2017; Candow et al., 2006, 2008; Holwerda et al., 2018; Krause et al., 2019; Leenders et al., 2013; Nabuco et al., 2018, 2019) administered MTN providing the three macronutrients (proteins, carbohydrates, and fats), where the protein source was milk concentrate, including whey and casein (Krause et al., 2019; Leenders et al., 2013) or just whey (Arnarson et al., 2013; Bell et al., 2017; Candow et al., 2006, 2008; Holwerda et al., 2018; Krause et al., 2019; Leenders et al., 2013; Nabuco et al., 2018, 2019). Only two studies included MTN with creatine monohydrate (CM), Candow et al. (2008) provided 0.1 g/kg, while by Bell et al. (2017) administered 2.5 g (~0.03 g/kg) of CM per dose. Furthermore, the admixture used by Bell et al. (2017) was the only one combining vitamin D, calcium, and n-3 PUFA.

Five studies administered the MTN using the following respective absolute supplement doses and energy content: 20 g and 169 Kcal (Arnarson et al., 2013), ~23 g and 93 Kcal (Leenders et al., 2013), 30 g and 116 Kcal (Bell et al., 2017), 20.7 g and 150 Kcal (Holwerda et al., 2018), and 27.1 g and 131 Kcal Nabuco et al. (2018, 2019). Conversely, the studies conducted by Candow et al. (2006) determined the doses based on the amount of protein per kg of body mass resulting in 0.30 g/kg with 2.1 kcal/kg and 0.34 g/kg with ~4.4 g/kg, respectively. On the other hand, Krause et al (2019), classified their participants into 4 categories according to their body mass (i) 45-59.9 kg; (ii) 60-74.9 kg (iii) 75-89.9 and (vi) 90-105 kg. The used MTN contained 72.7% of proteins and resulted in a consumption of about 8.7; 11.1; 13.6 and 16.1 g/kg of protein for the aforementioned four-category groups, respectively.

The supplementation protocol varied across studies. Leenders et al. (2013) and Arnarson et al. (2013) used a singular daily dose of the MTN or COMP administered after breakfast, or immediately post-workout respectively.

Candow et al. (2006) and Nabuco et al. (2018, 2019) used a two-daily dose supplementation protocol assigning participants to three groups differentiated by the moment in which the MTN or COMP was consumed: (i) MTN, pre-, and the COMP at post-workout, (ii) the COMP at pre- and the MTN at post-workout, and (iii) COMP at pre- and post-workout. Even though supplements were ingested twice, the MTN treatment involved only one intake per day (pre- or post-workout). No supplementation was administered on non-training days. Furthermore, Candow et al. (2008) tested a

3-dose protocol including supplements (MTN or COMP) at pre-, post-workout and before bedtime. Even though this study included three parallel groups: (i) MTN, (ii) COMP and (iii) creatine with sucrose, the latest was not included because the admixture included almost not protein but creatine and sucrose. Due to combining creatine with carbohydrates supplementation with physical training proven to significantly affect body composition and exercise performance per se (Cooper et al., 2012), no comparator using creatine was included. The studies by Bell et al (2017), Holwerda et al. (2018), and Krause et al. (2019), used a two-dose supplementation protocol. While Bell and colleagues (2017) supplemented their participants at breakfast and before bedtime only during training days, Holwerda et al. (2018) included supplements immediately post-workout and prior sleep time during training days while one dose (before bedtime) was implemented on non-training days. On the other hand, Krause et al. (2019) supplemented their participants on a daily based during breakfast and at midday.

Although no studies modified dietary intake, most of them recorded participants' dietary habits using either a 3-day (Arnarson et al., 2013, Bell et al., 2017; Candow et al., 2006, 2008; Holwerda et al., 2018), a 4-day dietary record intake (Leenders et al., 2013), or a 24-h dietary recall on two non-consecutive days such as in Nabuco et al. (2018, 2019). Data were collected at the beginning, during and final week of the intervention. No detailed information about dietary control was noted, advice to controls was only provided by Krause et al. (2019).

The impact of adding supplements (MTN and COMP) to the diet nutritional composition was reported in four of the selected studies. Candow et al. (2006), observed an increase in energy intake, albeit no changes in protein consumption due to the MTN or comparator ingestion was observed.

Bell et al. (2017) reported similar increases in energy for both MTN and COMP groups. Nonetheless, the MTN treatment increased protein intake from 1.1 to 1.6 g/kg/day. Holwerda et al. (2018) and Nabuco et al. (2018, 2019) reported significant increases in the daily protein intake, from 1.14 to 1.43; 0.92 to 1.38 and 0.94 to 1.49 g/kg/day, respectively for the participants allocated to the MTN treatment groups.

Body composition was assessed by Dual-energy X-ray absorptiometry (DXA) in seven studies (Arnarson et al., 2013; Bell et al., 2017; Holwerda et al., 2018; Krause et al., 2019; Leenders et al., 2013; Nabuco et al., 2018, 2019), while air-displacement plethysmography using BOD POD method was used by Candow et al. (2006, 2008).

Strength was assessed by the 1-RM bench press (Bell et al., 2017; Candow et al., 2006, 2008; Nabuco et al., 2018), leg press (Bell et al., 2017; Candow et al., 2006; Holwerda et al., 2018; Leenders et al., 2013) and leg extension (Bell et al., 2017; Holwerda et al., 2018; Leenders et al., 2013; Nabuco et al., 2018). Conversely, Bell et al. (2017) added up the 1 RMs values obtained by four upper body (horizontal row, chest press, lateral pull-down, and shoulder press) and two lower body (leg extension and leg press) exercises to assess regional strength changes. Additionally, the sum of all 1-RMs scores was considered a general marker of strength (Bell et al., 2017).

Krause et al. (2019) used an isometric handgrip strength test conducted on both hands while Arnarson et al. (2013) measured changes in quadriceps strength by a maximum voluntary isometric contraction test performed on an isokinetic dynamometer (Kin-Com 500H Chattanooga). The study by Nabuco et al. (2019) did not assess strength outcomes.

Functional capacity was assessed by a variety of testing protocols. All the included studies except those by Candow et al. (2006, 2008) used one of the following five tests to evaluate the ability to move and sustain fast movements involving mainly the lower limb musculature: The 5-times chair stand (Holwerda et al., 2018; Krause et al., 2019; Leenders et al., 2013; Nabuco et al., 2018), the 30-seconds chair stand (Bell et al., 2017; Krause et al., 2019), timed up and go (Arnarson et al., 2013; Bell et al., 2017), 4-meters walking test (Holwerda et al., 2018), 10-meters walking test (Krause et al., 2019; Nabuco et al., 2018). Only two studies (Arnarson et al., 2013; Bell et al., 2017) employed the 6-minute walk test.

Due to the similar nature of the tests, the 5-times chair stand (5STS) and the timed up-and-go (TUG) were merged with respect to data analysis. All other tests (30-seconds chair stand, 4-meters walking test, the 10-meters walking, 6-min walk test) were discussed separately.

Finally, two studies (Candow et al., 2006; Nabuco et al., 2018) conducted a 3-parallel arm design, with two different MTN supplementation protocols in each intervention (MTN intake pre- or post-workout). Consequently, these aforementioned studies were included and counted separately in the meta-analysed outcomes.

3.3. Changes on the analysed variables

3.3.1. Changes on Fat-Free Mass and Lean Body Mass

The estimated overall effect of MTN treatment vs. COMP was very small (n=10, *g*= 0.044, 95% CI −0.14 to 0.22). No significant heterogeneity was found within the 10 treatments [Q (9) = 6.025, p = 0.927, I^2 = 0]. As shown in Figure 2 no advantage for increasing FFM or LBM was observed when compared groups ingesting MTN to those groups consuming COMP.

Fig. 2

3.3.2. Changes in Muscular Strength

As described above, three studies measured upper body strength by the 1-RM score in the bench press. However, as the studies by Candow et al. (2006) and Nabuco et al. (2018) used a 3-arms parallel-group design (see table 1), 5 intervention groups were considered. As described in Figure 3, the estimated overall effect of MTN vs COMP was very small (n=5, *g*= 0.046, 95% CI −0.24 to 0.33) with no differences between groups. No significant heterogeneity [Q (4) = 0.532, p = 0.752, I^2 = 0] between treatments, was observed for changes measured in 1-RM bench press.

Fig. 3

Due to the nature of the outcomes, the two exercises used to assess lower-body strength, leg press (multi-joint) and leg extension (single joint) across studies were meta-analysed separately. As previously described, six publications assessed changes in lower-body strength, five (Bell et al., 2017; Candow et al., 2006, 2008; Holwerda et al., 2018; Leenders et al., 2013) used the 1-RM scores from two exercises, leg press (Figure 4) and leg extension (Figure 5). Consequently, five groups from leg press and leg extension were analysed. Furthermore, due to differences in the execution modality (isometric vs. dynamic action), and measurement criteria, data extracted from the study by Arnarson et al. (2013) were not meta-analysed but still discussed separately.

As described by Figure 4, the estimated overall effect of MTN vs. COMP was very small (n=5, *g*= 0.025, 95% CI -0.26 to 0.31). Additionally, no significant heterogeneity [Q (4) = 4.011, p = 0.629, I^2 = 0.280] was observed between treatments for the changes measured in 1-RM leg press.

Fig. 4

Similarly, Figure 5 shows that the estimated overall effect of MTN vs COMP was very small for the changes measured in 1-RM leg extension (n=5, g= 0.106, 95% CI -0.15 to 0.36). Additionally, no significant heterogeneity was observed [Q (4) = 2.341, p = 0.106, I^2 = 0] between treatments.

Fig. 5.

3.3.3. Changes in Functional Capacity

As previously described, the two tests (5STS and TUG) focused on the ability to move and sustain fast movements were analysed together. Although six studies assessed the functional capacity, Nabuco et al. (2018) assessed functional capacity by the 5STS in two different MTN-based intervention groups ingesting the supplement before (MTN-COMP) or after (COMP-MTN) workouts, therefore 7 groups were included in the analysis. As described in Figure 6, no significant differences between MTN and COMP (g=0.079, 95% CI -0.12 to 0.27) were observed. Additionally, no significant heterogeneity [Q (6) = 4.538, p = 0.574, I^2 = 0] was observed between treatments.

Fig. 6.

When considering the impact of using MTN or COMP added to exercise interventions on functional capacity measured by alternative assessment protocols, the observed results are in line with the performed meta-analysis including only the two (5STS and TUG) selected tests. For instance, Holwerda et al. (2018) reported no time (p=0.604), treatment (p=0.273) or time interaction (p=0.877) effects of adding MTN or COMP to a 12-week resistance training protocol on the time to complete the 4-Meters walking test in a group of older men. Similarly, Krause et al. (2019), reported a significant time effect (about 30% improvement) in both treatments, MTN and COMP but with no significant (p>0.05) treatment effect after a 12-week intervention period. Furthermore, Bell et al. (2017) did not find a time or treatment effect on the 30-Seconds sit-to-stand test after 12 weeks. Additionally, two studies (Arnarson et al., 2013; Bell et al., 2017) used the 6-minutes walking test, and although significant post-treatment improvements for both MTN and COMP were identified, none of the studies reported differences between groups. Conversely, Nabuco et al. (2018) was the only study reporting a significant interaction effect, favouring MTN vs. COMP to reduce the time to complete the 10-Meters walking test. In this study, the two MTN groups consuming the supplement either before (MTN-pre) or after (MTN-post) workout significantly improved (10.8% and 11.8%, respectively) compared to the COMP group.

3.4. Synthesis of results

The Grading of Recommendations, Assessment, Development and Evaluation (GRADE) Working Group approach has been followed to summarise the evidence and assess the quality of the evidence factors. The results show a very low level of evidence in the estimated effect for the inclusion of MTN when compared to COMP in healthy middle-aged and older adults in every analysed outcome.

3.5. Additional Analyses

No subgroup analysis was performed to differentiate between supplementation timing (breakfast, pre- or post-workout, before bedtime,) as insufficient differences between results were observed (I^2=0).

The studies of Candow et al. (2006) and Nabuco et al. (2018) used a three-parallel-group randomised design. From these studies, two different treatment protocols tested the effects of ingesting MTN before and COMP post-workout, compared to the ingestion of COMP pre-workout and MTN post-workout, against the ingestion of COMP at both pre-and post-workout. Thus, two diverse treatments involving the intake of MTN could be considered from each study because of supplement intake and timing variation.

3.6. Outliers and Publication Bias

No studies were identified as outliers for any of the assessed outcomes. Funnel plots (see Supplementary Material Figures S2, S3, S4, S5 and S6) describe a practically symmetrical plot, the “trim and fill” procedure resulted in an overall treatment effect of −0.04 for the Fat-Free Mass, and when 1 study was added to the left of the mean, it reduced the effect to 0.02. A similar tendency was observed for upper body strength, with a treatment effect of 0.05, which increased up to 0.09 when 2 studies were added to the right of the mean. Also, a positive result of 0.02 was observed for leg press, with no studies added to any side of the mean, and higher values for the leg extension, which resulted in 0.11 and increasing to 0.13 when one study was added to the right of the mean. Finally, the analysis of the functional capacity showed a result of 0.08 turning into 0.04 when one study was added to the left of the mean (Figure S6).

4. Discussion

Compared to the ingestion of an isocaloric nutritional comparator, no additional benefits on any of the investigated training outcomes (body composition, muscle strength, and functional capacity) were observed by the administration of a protein-based MTN.

Our results differ from two previous systematic reviews including adults ≥ 60 years old (Liao et al., 2017) or adults >45 years old (O'Bryan et al., 2019). Liao et al. (2017) observed beneficial effects of adding protein-based supplementation to optimise gains of lean mass and strength in overweight or obese, ≥60 years old individuals following resistance training programmes. However, though Liao et al. (2017) compared the effects of combining resistance training with either protein-based or non-protein supplements, these authors included groups with no supplementation (merely resistance training). In our review, only studies including isoenergetic non-protein comparators added to any kind of exercise intervention were considered. As previously highlighted, regardless of macronutrient composition, energy intake has proven to be one of the most relevant nutritional factors impacting training-induced outcomes (e.g., strength gains or hypertrophy) (Naclerio and Larumbe-Zabala, 2016). Furthermore, Liao et al. (2017), considered overweight and obese individuals undergoing resistance training, while we restricted our analysis to middle-aged and older healthy non-obese participants. As indicated by the authors, participants with a BMI >30 exhibited substantially greater lean mass and strength gains in response to protein-based supplementation, suggesting that combining resistance exercises with protein supplementation administered in isolation or as part of a MTN formulation elicited a more favourable effect in overweight or obese individuals (Liao et al., 2017). Only high-quality protein sources such as milk protein concentrate (Krause et al., 2019; Leenders et al., 2013) or whey were administered in the MTN formulations in the nine included studies. Except for the study by Bell et al. (2017), which mostly excluded carbohydrates from the MTN, the rest of the included interventions combined high-quality protein and carbohydrates. Even though combining protein and carbohydrates in a single intake has been proposed as an effective strategy to maximise resistance training outcomes in young athletes (Jäger et al. 2017), our results do not show any additional benefit from this combination in middle-aged and older healthy non-obese participants. In fact, the results by Bell et al (2017) do not seem to impact the lack of effects observed on the analysed variables (figures 2 to 6).

High-quality protein sources with a higher amount of leucine (>6 to 12%) e.g., whey, have proven to enhance muscle protein synthesis under exercise as well as in resting conditions in both young and elderly individuals (Moore, 2021). Overweight and obese older people, even when physically active, may be at greater risk of losing lean mass compared to normal-weight physically active controls (Liao et al., 2017), and therefore may benefit even greater from adding MTN-based protein supplementation to significantly improve muscle mass and strength from resistance training programmes. It is worth noticing that similar to our results, Liao et al. (2017) failed to observe additional benefits of protein-based supplementation to improve functional capacity assessed by the same two tests used in our review (the timed up-and-go and the chair rise time test). The nature of the physical tasks emphasising a higher neuromuscular activation and movement coordination over a relatively short period of time (<30 sec) reduced the relevance of nutrition in supporting the outcomes as observed in strength and muscle mass gain by Liao and colleagues. Nonetheless, it is worth noticing that resistance training provides the most efficient anabolic stimulus for skeletal muscle tissue growth and strength improvement (Morton et al., 2018). Therefore, in well-nourished individuals, as it was the case of all studies included in our review, the lack of effect of adding MTN-based protein to maximise lean mass, muscle strength, and functional capacity, supports the notion that energy supply, rather than the macronutrient proportion, is likely the most important nutritional factor impacting performance adaptation (McLellan et al., 2014).

O'Bryan et al. (2019) reported significant beneficial effects of combining resistance training with the ingestion of MTN vs. a comparator group ingesting either placebo, only protein, or performing only resistance training (with no supplement) on improving FFM or LBM and strength performance in older adults (>45 years old). Differences in the inclusion criteria precluded us to include some studies analysed by O'Bryan and colleagues. For instance, the studies by Rondanelli et al. (2016), Seino et al. (2018) and Carter et al. (2005) favoured the effects of MTN over placebo to induce greater gains of FFM or LBM in the review by O'Bryan et al. (2019). These studies were not included in our analysis. Specifically, Rondanelli et al. (2016) studied sarcopenic patients, the study by Seino et al. (2018) was excluded due to the lack of an isocaloric COMP while the study by Carter et al. (2005), besides using non-isocaloric supplements (MTN provided more than 120 kcal per serving than the COMP) reported incomplete FFM data. Similarly, when looking at upper and lower body strength, two studies (Bemben et al., 2010; Villanueva et al., 2014) impacted the results favouring MTN benefits over COMP in the O'Bryan et al. (2019) review. Both studies were excluded from the present meta-analysis due to the lack of an isocaloric control group.

Another factor influencing our results is the amount of daily protein intake. Except for the study of Bell et al. (2017) where participants in the MTN treatment ingested a significantly higher amount of protein per day with respect to the COMP group [1.7 ± 0.5 (6 weeks) or 1.6 ± 0.5 (19 weeks) vs. 1.2 ± 0.3 g/kg), no significant differences were reported for the other eight studies. Indeed, the average daily protein intake of both groups (MTN or COMP) across studies was still inferior to 1.6 g/kg, which is currently considered the optimal protein ingestion in physically active adults and beyond which protein supplementation ceases to provide a measurable benefit in maximising resistance exercise outcomes (Morton et al., 2018). Accordingly, it could have been expected that the addition of MTN-based protein instead of a non-protein isocaloric supplement enhanced training outcomes in the intervention groups. Furthermore, the aforementioned study of Bell and colleagues, the only one providing protein about 1.6 g/kg/d or higher, reported significant improvement in MTN vs. COMP only for upper body strength while no differences were determined in lower body strength and FFM (Table 1). Taken together, it seems that as long as the daily energy intake is equated, the addition of an MTN-protein-based supplement without reaching the recommended daily protein intake of 1.6 g/kg BM but still approaching the estimated average requirement of 1.24 (Moore 2021) induced no further benefits in the investigated training outcomes.

Although older trained adults are unlikely to suffer from the typical age-related anabolic resistance (Moore, 2021), most of the participants of the analysed studies were considered untrained or slightly physically active, and therefore it is expected to have a reduced anabolic response requiring higher per-meal protein doses. This would result in a higher daily protein intake to achieve similar rates of muscle protein synthesis compared with younger individuals, as protein is the primary variable regulating changes in skeletal muscle mass (Rennie et al., 2004). In fact, protein-based supplementation seems to be less effective with increasing chronological age (Morton et al., 2018). Thus, the lack of response to MTN supplementation to maximise training outcomes in older individuals suggests they may have an increased need for higher protein intake reaching at least 1.6 g/kg/d and support the notion that training is the most efficient stimulus to increase exercise performance lean mass.

In summary, the pooled estimates from the present study show MTN supplementation during prolonged RT (≥ 6 weeks) did not promote additional benefits to augment gains in body composition (i.e., FFM or LBM), upper and lower body strength, or functional capacity when compared with isocaloric non-MTN treatments. MTN formulations employed by the nine studies included in the present review included milk protein concentrate and whey protein combined with other synergistic anabolic compounds such as creatine monohydrate (Bell et al., 2017; Candow et al., 2008), or leucine (Holwerda et al., 2018) and additional macronutrients/micronutrients including CHO, vit D and PUFAs (Bell et al., 2017).

4.1. Limitations and recommendation for future studies

Several aspects of this review must be considered when attempting to draw evidence-based inferences. The small number of treatments included in this review represent an important limitation. We identified some potential sources of heterogeneity across the included studies: (i) the supplement composition (e.g., including creatine), (ii) the timing of the protocol (e.g., at breakfast vs. pre- and post-workout), (iii) dose consumed (absolute vs. relative to body composition intakes), (iv) duration of the intervention (range of 8 to 24 weeks) and (v) the configuration of the training workouts (e.g., using free weights, machines, or elastic bands, 4 to 11 exercises, 2–6 sets per exercise and 30 sec to 2 min rest with different patterns of training load progressions across studies), potentially impacting the observed lack of benefits of ingesting MTN vs. an isocaloric non-protein containing comparator. Furthermore, although this review aimed to assess the effectiveness of MTN combined with exercise in middle-aged and older adults (≥45 years old), most of the analysed interventions were conducted with older adults (>65 yrs.). Therefore, there is still a paucity of research on the effectiveness of MTN supplementation in middle-aged physically active adults. Additionally, given the potential benefits of protein supplementation alone to maximise-exercise induced outcomes, particularly lean body mass in older adults (Vieira et al. 2022), studies comparing MTN vs. protein mono-component are necessary.

In summary, studies using a broad range of middle-aged participants, longer intervention periods (>24 weeks), stricter control of diet and supplementation protocol comparing MTN vs. non-protein and only protein-containing comparators are necessary to fully understand the role of combining MTN with exercise to optimise physical training adaptation in healthy older adults.

5. Conclusions

The available evidence from RCTs suggests no additional benefits are obtained by combining exercise intervention with the ingestion of a MTN instead of isocaloric comparator on body composition, strength, and functional capacity outcomes in healthy physically active middle-aged and older adults.

Authorship:

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Joel Puente-Fernandez, Fernando Naclerio and Eneko Larumbe-Zabala. The first draft of the manuscript was written by Fernando Naclerio and Joel Puente-Fernandez and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Conflicts of interest:

None of the authors declare to have any conflicts of interest relevant to the information provided in this review.

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Figures Captions

Figure 1. PRISMA-P Flow chart diagram of the study selection. MPS: Muscle Protein Synthesis; COMP: Isocaloric Comparator

Figure 2. Fat-free mass or lean body mass Forest-plot. Results of a random-effects meta-analysis showed the effect size (g) with 95% confidence interval. The black diamond represents the pooled (overall) standardised mean difference. CI: confidence interval, *Blank: No subgroup analysis or intervention; COMP: Comparator; MTN: Multi-ingredient Supplement; MTN-A: MTN intake after training session; MTN-B: MTN intake before training session*

Figure 3. Upper body Strength Forest-plot. Results of a random-effects meta-analysis showed the effect size (g) with 95% confidence interval. The black diamond represents the pooled (overall) standardised mean difference. CI: confidence interval, Blank: No subgroup analysis or intervention; COMP: Comparator; MTN: Multi-ingredient Supplement; MTN-A: MTN intake after training session; MTN-B: MTN intake before training session.

Figure 4. Lower Body (Leg Press) Strength Forest-plot. Results of a random-effects meta-analysis showed the effect size (g) with 95% confidence interval. The black diamond represents the pooled (overall) standardised mean difference. CI: confidence interval, Blank: No subgroup analysis or intervention; COMP: Comparator; MTN: Multi-ingredient Supplement; MTN-A: MTN intake after training session; MTN-B: MTN intake before training session.

Figure 5. Lower Body (Leg Extension) Strength Forest-plot. Results of a random-effects meta-analysis showed the effect size (g) with 95% confidence interval. The black diamond represents the pooled (overall) standardised mean difference. CI: confidence interval, Blank: No subgroup analysis or intervention; COMP: Comparator; MTN: Multi-ingredient Supplement; MTN-A: MTN intake after training session; MTN-B: MTN intake before training session.

Figure 6. Functional Capacity (Timed Up-and-go and 5 Times Sit-to-Stand) Forest-plot. Results of a random-effects meta-analysis showed the effect size (g) with 95% confidence interval. The black diamond represents the pooled (overall) standardised mean difference. CI: confidence interval, 5STS: 5-Times sit-to-stand test; Blank: No subgroup analysis or intervention; COMP: Comparator; MTN: Multi-ingredient Supplement; MTN-A: MTN intake after training session; MTN-B: MTN intake before training session; TUG: Timed up-and-go test.

Table 1. Summary of the randomized controlled trials included in the meta-analysis

Study	Participants	Supplementation Design[a]	Length	Training protocol	Supplementation protocol	Outcomes
Candow et al. (2006)	Males, n=29; age 59-76; UT	3PG: (i) MTN-B (n=9); (ii) MTN-A (n=10); (iii) COMP (Maltodextrin/Sucrose, n=10)	12 wk	RT, three days/wk, 9 ex x 3 sets x 10 reps @70% or 10 RM; with 2 mins rest	Two doses (~2.1 kcal/kg each): one at pre- and one at post-workout. MTN: 0.54 g/kg; COMP: 0.63 g/kg	↔FFM[b] ↔UBS 1RM[b] ↔LBS 1RM[b]
Candow et al. 2008)	Males, n=35; age 59 to 77; UT	3PG: (i) MTN (n=10); (ii) Creatine-Sucrose (n=13) EXCLUDED (ii) COMP (Sucrose, n=12)	10 wk	RT, three days/wk, 9 ex, 3 sets of 10 reps @70% or 10 RM; with 2 mins rest	Three Equal doses (~0.4 g/kg each ~1.6 kcal/kg): one at pre-, one post-workout, and one prior to bedtime	↑FFM[c] ↑UBS 1RM[c] ↔LBS 1RM[b]
Arnarson et al. (2013)	Males (n=67) and females (n=94); n=141; age 65 to 91; UT	2PG: (i) MTN (n=75); (ii) COMP (Carbohydrate and 1 g of fat, n=66)	12 wk	PRT three days/wk; 2 to 3 sets, 10 reps @60%; 3 sets 6-8 reps @75-80%	One dose of 51 g (169 kcal) mixed with 250 ml of water at postworkout	↔FFM[b] ↔LBS[b] ↔TUG[b]

				1RM. The load increased 5-10% per week		
Leenders et al. (2013)	Males (n=29) and Females (n=24); n=53; age 70±1; recreationally active participants	2PG: (i) MTN (n=21); (ii) COMP (Lactose and Calcium, n=20)	24 wk	PRT. three days/wk: 6 ex, 3-4 sets, 10-15-8-10 reps @60-75 or 80% 1RM; with 1.5 to 3 mins rest	MTN (93 kcal) and COMP (28 kcal) consumed daily after breakfast.	↑FFM[b] ↑%FM[b] ↑LBS 1RM[b] ↑5ChSt[b]
Bell et al. (2017)	Males, n=41; age 73±1;UT	2PG: (i) MTN (n=21); (ii) COMP (Carbohydrate, n=20)	12 wk	PRT: two days/wk, 3 sets, 6 ex, 10-12 reps @70-80%. HIIT (1 day): 10 reps of 60 s @90% HR max	Two doses of 116 (MTN) and 56 (COMP) kcal: one after breakfast and one prior bedtime	↔FFM[b] ↑TUG[b] ↑LBS[b] ↑UBS[c]

				with 1 min rest		
Holwerda et al. (2018)	Males, n=41; age 70±1; UT	2PG: (i) MTN (n=21); (ii) COMP (Carbohydrate and fat, n=20)	12 wk	PRT, three days/wk: 4 ex per workout 2-4 sets, 8-10 reps @ 70-80% 1RM with 2-3 mins rest	Training days: Two doses (150 kcal each), one at post-workout, and one prior to bedtime. Non-training days: One dose prior to bedtime	↔FFM[b] ↑LBS 1RM[b] ↑5ChSt[b]
Nabuco et al. (2018)	Females, n=66; age 67±7; UT	3PG: (i) MTN-COMP (n=22) (ii) COMP-MTN (n=21); (iii) COMP-COMP (Maltodextrin, n=23)	12 wk[e]	PRT, three days/wk: 8 ex, 3 sets, 10 to 8-12 RM. The load increased weekly	Training days: two doses (~130 kcal each), one at pre- and one at post-workout	↑FFM[c] ↑UBS 1RM[c] ↑LBS 1RM[c]
Krause et al. (2019)	Males (n=9) and females (n=12), n=21; age 64±4; UT	2PG: MTN (n=11); (ii) COMP (Maltodextrin, n=10)	12 wk	PRT with Elastic Bands: 11 ex, 3 days/wk: 4-6 sets, 8-15 reps with 30 s	Two doses of ~0.8 kcal/kg: one at breakfast and one at midday	↑FFM[b] ↑%FM[c] ↑5ChSt[b]

				rest		
Nabuco et al. (2019)	Females, n=66; age 67±7; UT	3PG: (i) MTN-COMP (n=22) (ii) COMP-MTN (n=21); (iii) COMP-COMP (Maltodextrin, n=23)	8 wk	PRT, three days /wk: 8 ex, 3 sets, 10 to 8-12 RM. The load increased weekly	Training days: Two doses of 35 g (~140 kcal each), one at pre- and one at post-workout	↑ FFM[b, d] ↑ FM[e]

Notes: 5ChSt: 5-times chair sit to stand test**; CHO**: carbohydrates**; ex**: exercises**; EXCLUDED**: the group consuming creatine monohydrate with negligible amount of proteins and carbohydrate was excluded from the analysis (see text for further explanations); **FFM**: fat free mass; **FM**: fat mass**; LBS**: Lower Body Strength; **MTN**: multi-ingredient**; MTN-B**: MTN was ingested at pre-workout and comparator at post-workout; **MTN-A**: was ingested at post-workout and comparator at pre-workout; **PG:** parallel groups; **PRT:** progressive resistance training; **reps**: repetitions; **RM:** maximum number of repetitions per set; **TUG**: timed up-and-go test; **UBS**: Upper Body Strength; **UT**: untrained; **wk**: weeks.

Symbols: [a]Even though all multi-ingredient and comparator supplements matched the inclusion criteria, their composition differs between studies; ↑ = significant improvement to baseline; ↔ = no differences to baseline; [b] No differences ($p>0.05$) between MTN vs. COMP; [c] Differences ($p<0.05$) between MTN vs. COMP; [d] Differences ($p<0.05$) between MTN-PLA vs. PLA-MTN and MTN-PLA vs. COMP; [e] Differences ($p<0.05$) between PLA-MTN vs. COMP.

Figure 1

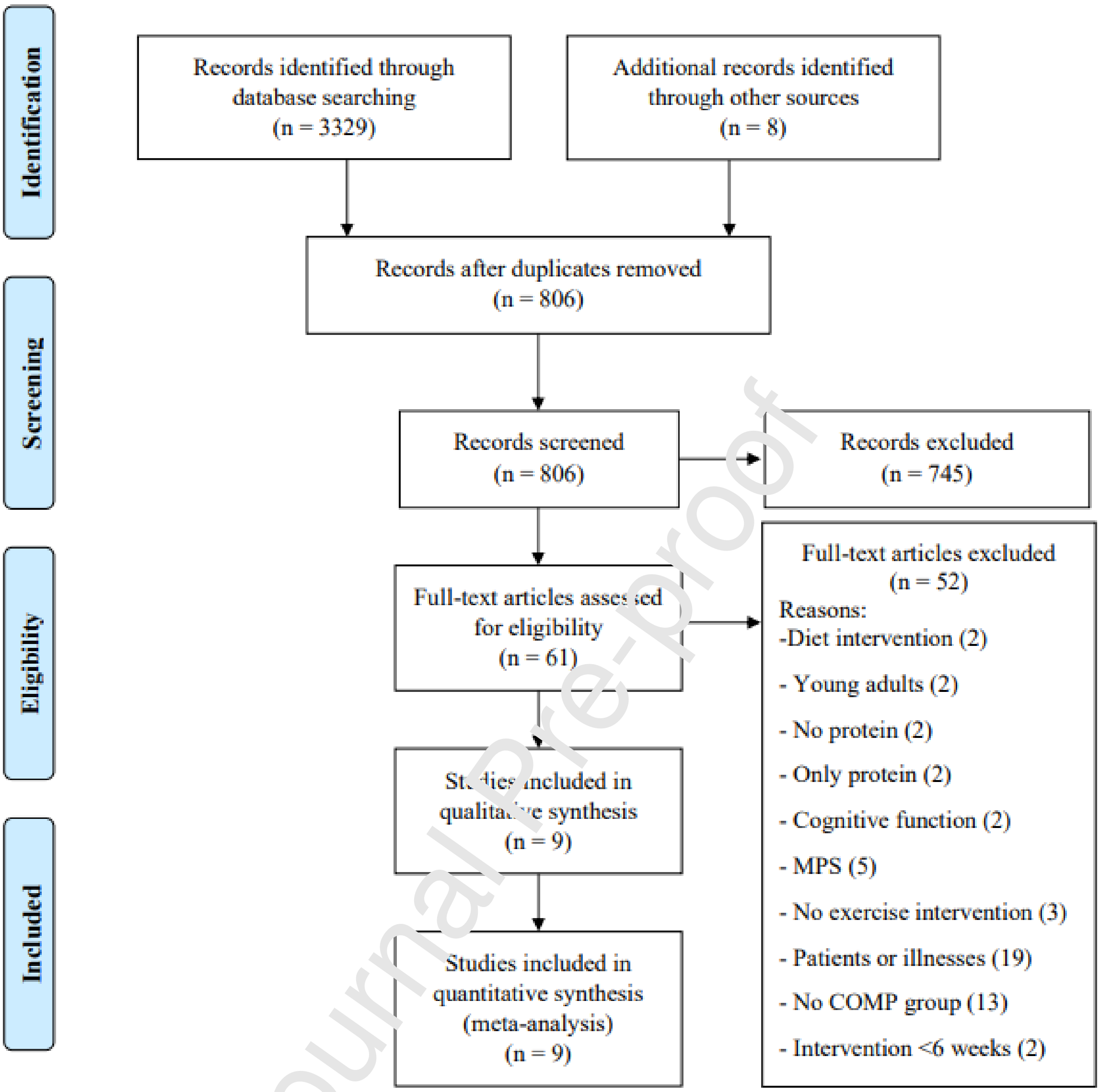
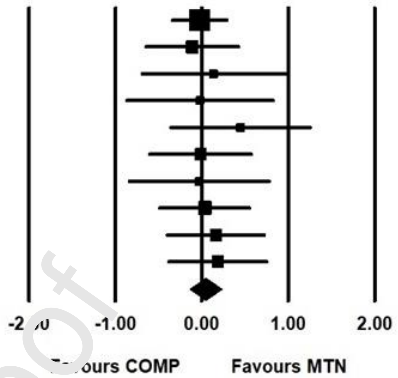

Identification
Screening
Eligibility
Included
Records identified through database searching (n = 3329)
Additional records identified through other sources (n = 8)
Records after duplicates removed (n = 806)
Records screened (n = 806)
Records excluded (n = 745)
Full-text articles assessed for eligibility (n = 61)
Full-text articles excluded (n = 52)
Reasons:
-Diet intervention (2)
- Young adults (2)
- No protein (2)
- Only protein (2)
- Cognitive function (2)
- MPS (5)
- No exercise intervention (3)
- Patients or illnesses (19)
- No COMP group (13)
- Intervention <6 weeks (2)
Studies included in qualitative synthesis (n = 9)
Studies included in quantitative synthesis (meta-analysis) (n = 9)

Figure 2

Study name	Subgroup within study	Statistics for each study			Sample size	
		Hedges's g	Lower limit	Upper limit	MTN	COMP
Arnarson et al. (43)		-0.023	-0.35	0.31	75	66
Bell et al. (19)		-0.111	-0.66	0.44	25	24
Candow et al. (15)	MTN-A	0.136	-0.70	0.98	10	10
Candow et al. (15)	MTN-B	-0.019	-0.88	0.84	9	10
Candow et al. (38)		0.448	-0.37	1.27	10	12
Holwerda et al. (39)		-0.012	-0.61	0.59	21	20
Krause et al. (42)		-0.030	-0.85	0.79	11	10
Leenders et al. (44)		0.034	-0.50	0.56	27	26
Nabuco et al. (40)	MTN-COMP	0.164	-0.41	0.74	22	23
Nabuco et al. (40)	COMP-MTN	0.186	-0.40	0.77	21	23
		0.044	-0.14	0.22		

Hedges's g and 95% CI

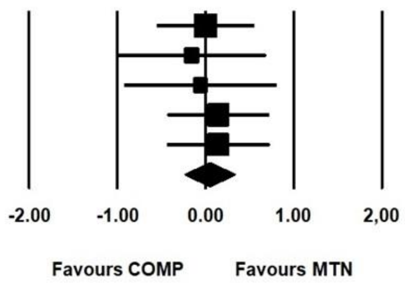


Meta Analysis

Figure 3

Study name	Subgroup within study	Hedges's g	Lower limit	Upper limit	MTN	COMP
		Statistics for each study			Sample size	
Bell et al. (19)		0.000	-0.55	0.55	25	24
Candow et al. (15)	MTN-A	-0.159	-1.00	0.68	10	10
Candow et al. (15)	MTN-B	-0.058	-0.92	0.80	9	10
Nabuco et al. (40)	MTN-COMP	0.144	-0.43	0.72	22	23
Nabuco et al. (40)	COMP-MTN	0.144	-0.44	0.73	21	23
		0.046	-0.24	0.33		

Hedges's g and 95% CI

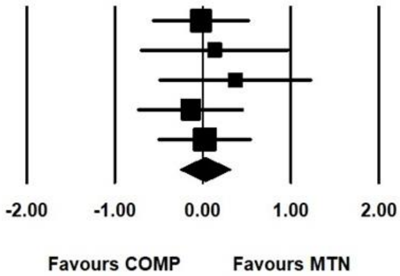


Meta Analysis

Figure 4

Study name	Subgroup within study	Hedges's g	Lower limit	Upper limit	MTN	COMP
		Statistics for each study			Sample size	
Bell et al. (19)	LP	-0.021	-0.57	0.53	25	24
Candow et al. (15)	LP-MTN-A	0.134	-0.71	0.97	10	10
Candow et al. (15)	LP-MTN-B	0.371	-0.50	1.24	9	10
Holwerda et al. (39)	LP	-0.137	-0.74	0.46	21	20
Leenders et al. (44)	LP	0.023	-0.51	0.55	27	26
		0.025	-0.26	0.31		

Hedges's g and 95% CI

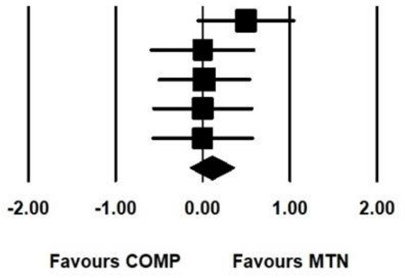


Meta Analysis

Figure 5

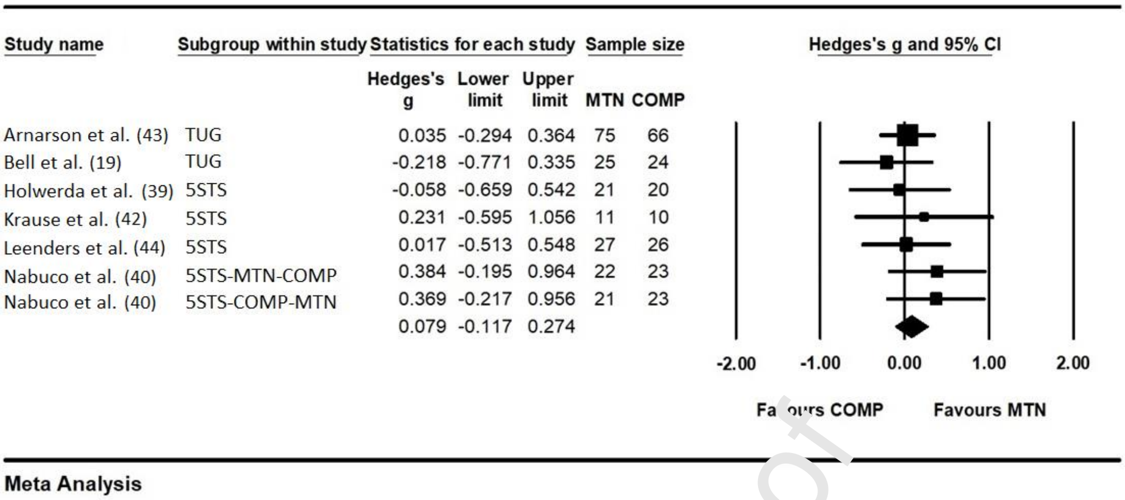
Study name	Subgroup within study	Statistics for each study: Hedges's g	Lower limit	Upper limit	Sample size: MTN	COMP
Bell et al. (19)		0.496	-0.06	1.06	25	24
Holwerda et al. (39)		0.000	-0.60	0.60	21	20
Leenders et al. (44)		0.019	-0.51	0.55	27	26
Nabuco et al. (40)	LE-MTN-COMP	0.000	-0.57	0.57	22	23
Nabuco et al. (40)	LE-COMP-MTN	0.000	-0.58	0.58	21	23
		0.106	-0.15	0.36		

Hedges's g and 95% CI



Meta Analysis

Figure 6


Study name
Subgroup within study
Statistics for each study
Sample size
Hedges's g and 95% CI
Hedges's g
Lower limit
Upper limit
MTN
COMP
Arnarson et al. (43) TUG 0.035 -0.294 0.364 75 66
Bell et al. (19) TUG -0.218 -0.771 0.335 25 24
Holwerda et al. (39) 5STS -0.058 -0.659 0.542 21 20
Krause et al. (42) 5STS 0.231 -0.595 1.056 11 10
Leenders et al. (44) 5STS 0.017 -0.513 0.548 27 26
Nabuco et al. (40) 5STS-MTN-COMP 0.384 -0.195 0.964 22 23
Nabuco et al. (40) 5STS-COMP-MTN 0.369 -0.217 0.956 21 23
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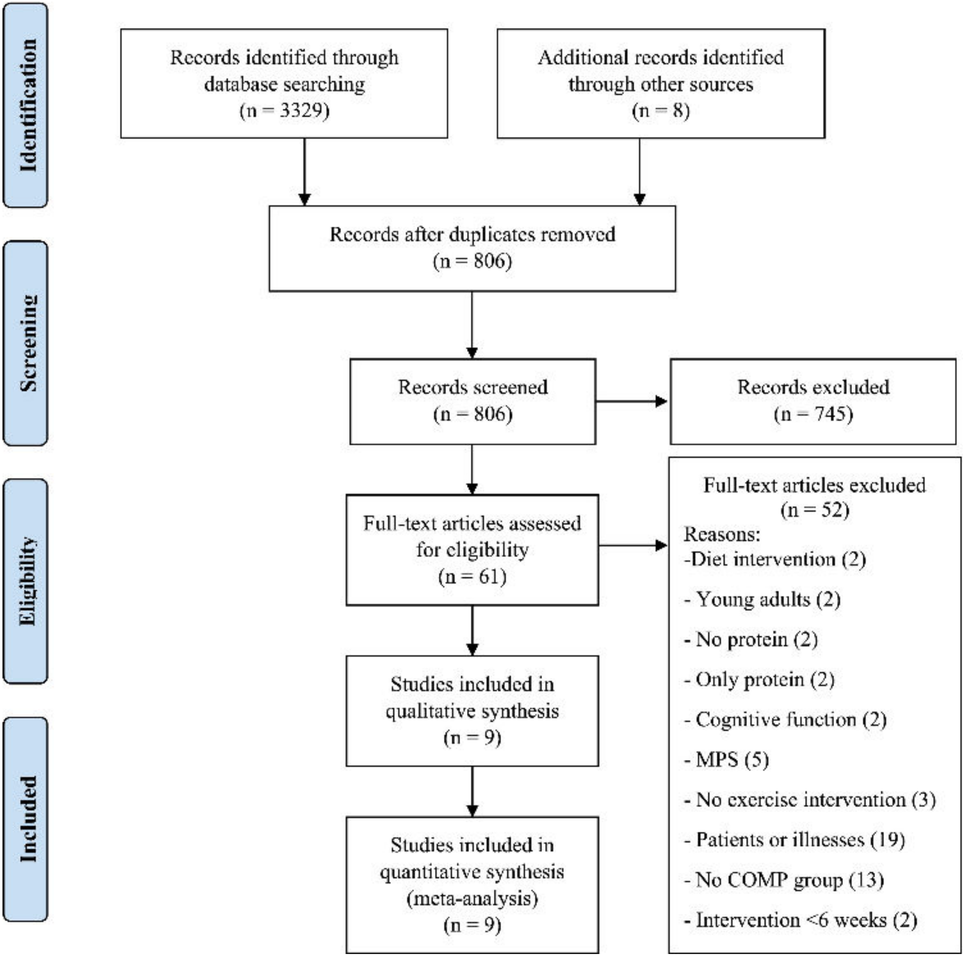
0.00
1.00
2.00
Favours COMP
Favours MTN
Meta Analysis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-Diet intervention (2)
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Studies included in qualitative synthesis (n = 9)
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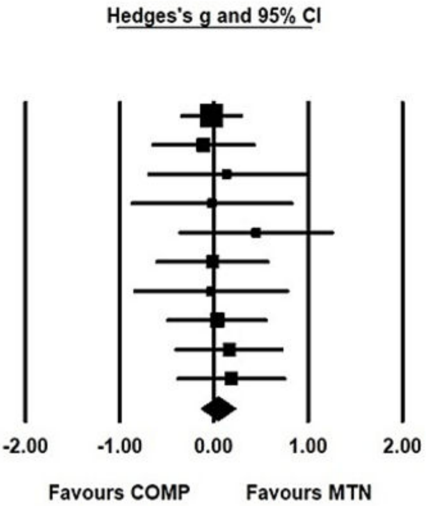
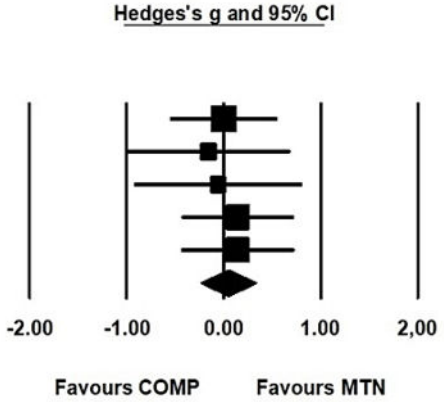
		0.044	-0.14	0.22		



Meta Analysis

Figure 2

Study name	Subgroup within study	Statistics for each study			Sample size	
		Hedges's g	Lower limit	Upper limit	MTN	COMP
Bell et al. (19)		0.000	-0.55	0.55	25	24
Candow et al. (15)	MTN-A	-0.159	-1.00	0.68	10	10
Candow et al. (15)	MTN-B	-0.058	-0.92	0.80	9	10
Nabuco et al. (40)	MTN-COMP	0.144	-0.43	0.72	22	23
Nabuco et al. (40)	COMP-MTN	0.144	-0.44	0.73	21	23
		0.046	-0.24	0.33		

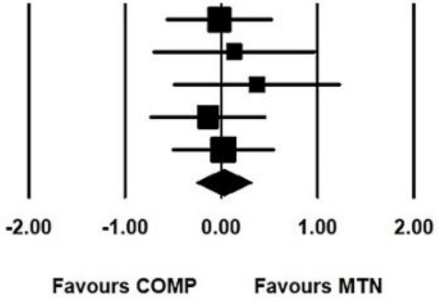


Meta Analysis

Figure 3

Study name	Subgroup within study	Statistics for each study: Hedges's g	Lower limit	Upper limit	Sample size: MTN	COMP
Bell et al. (19)	LP	-0.021	-0.57	0.53	25	24
Candow et al. (15)	LP-MTN-A	0.134	-0.71	0.97	10	10
Candow et al. (15)	LP-MTN-B	0.371	-0.50	1.24	9	10
Holwerda et al. (39)	LP	-0.137	-0.74	0.46	21	20
Leenders et al. (44)	LP	0.023	-0.51	0.55	27	26
		0.025	-0.26	0.31		

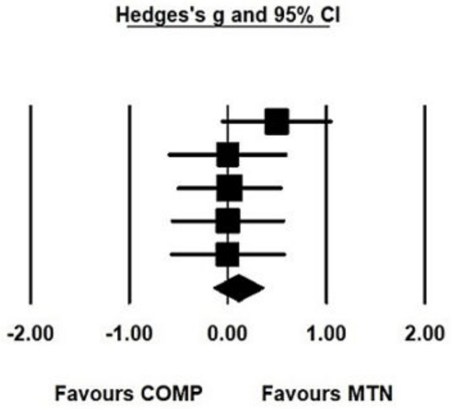
Hedges's g and 95% CI



Meta Analysis

Figure 4

Study name	Subgroup within study	Statistics for each study			Sample size	
		Hedges's g	Lower limit	Upper limit	MTN	COMP
Bell et al. (19)		0.496	-0.06	1.06	25	24
Holwerda et al. (39)		0.000	-0.60	0.60	21	20
Leenders et al. (44)		0.019	-0.51	0.55	27	26
Nabuco et al. (40)	LE-MTN-COMP	0.000	-0.57	0.57	22	23
Nabuco et al. (40)	LE-COMP-MTN	0.000	-0.58	0.58	21	23
		0.106	-0.15	0.36		

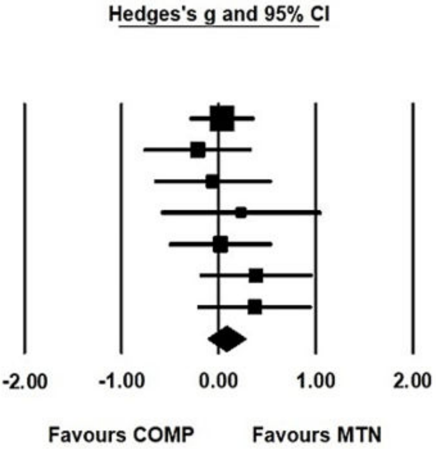


Meta Analysis

Figure 5

Study name	Subgroup within study	Hedges's g	Lower limit	Upper limit	MTN	COMP
Arnarson et al. (43)	TUG	0.035	-0.294	0.364	75	66
Bell et al. (19)	TUG	-0.218	-0.771	0.335	25	24
Holwerda et al. (39)	5STS	-0.058	-0.659	0.542	21	20
Krause et al. (42)	5STS	0.231	-0.595	1.056	11	10
Leenders et al. (44)	5STS	0.017	-0.513	0.548	27	26
Nabuco et al. (40)	5STS-MTN-COMP	0.384	-0.195	0.964	22	23
Nabuco et al. (40)	5STS-COMP-MTN	0.369	-0.217	0.956	21	23
		0.079	-0.117	0.274		

(Column groups: Statistics for each study — Hedges's g, Lower limit, Upper limit; Sample size — MTN, COMP)



Meta Analysis

Figure 6