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The role of protein intake and its timing on body composition and muscle function in healthy adults: a systematic review and meta-analysis of randomized controlled trials

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Running title: Effect and timing of protein intake

Abbreviations used: 1-RM, one-repetition maximum; BMI, body mass index; CSA, cross-sectional muscle area; LBM, lean body mass; LP, low-protein diet; MD, mean difference; MT, muscle thickness; NP, no protein supplementation; PLA, non-protein placebo.

Abstract

Background: Increased protein intake has been suggested to improve gains in muscle mass and strength in adults. Furthermore, the timing of protein intake has been discussed as a margin of opportunity for improved prevention measures.

Objective: This systematic review investigated the effect of protein supplementation on body composition and muscle function (strength and synthesis) in healthy adults, with an emphasis on the timing of protein intake.

Methods: Randomised controlled trials were identified using Pubmed, Web of Science, CINAHL and Embase, up to March 2019. For meta-analyses, data on lean body mass (LBM), handgrip strength and leg press strength were pooled by age group (mean age 18-55 or >55 years) and timing of protein intake. The quality of evidence was assessed using GRADE approach.

Results: Data from 65 studies with 2,907 participants (1514 men and 1380 women, 13 unknown sex) were included in the review. Twenty-six, eight and 24 studies were used for meta-analysis on LBM, handgrip strength and leg press strength, respectively. The protein supplementation was effective in improving (mean difference; 95% CI) LBM in adults (0.62 kg; 0.36, 0.88) and older adults (0.46 kg; 0.23, 0.70), but not handgrip strength (older adults: 0.26 kg; -0.51, 1.04) and leg press strength (adults: 5.80 kg; -0.33, 11.93, older adults: 1.97 kg; -2.78, 6.72). Sensitivity analyses removing studies without exercise training had no impact on the outcomes. Data about muscle synthesis were scarce and inconclusive. Subgroup analyses showed no beneficial effect of a specific timing of protein intake on LBM, handgrip strength and leg press strength.

Conclusion: Overall, the results support the positive impact of protein supplementation on LBM of adults and older adults, independently of its timing of intake. Effects on muscle

26 strength and synthesis are less clear and need further investigation. This systematic review was
27 registered on PROSPERO as CRD42019126742.

28

29 **Keywords:** protein, timing of intake, body composition, lean body mass, muscle strength,
30 muscle synthesis, adults, elderly, obese

Introduction

Higher total protein intake ($1.2\text{--}1.5\text{ g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$) is proposed to help preserve lean body mass (LBM) and improve body composition during weight loss in adults (1-4). Resistance exercise training, in combination with dietary protein supplementation, has been promoted as an effective approach to attenuate the loss in muscle mass with age (3). However, disagreements still exist about the protein supplementation - some of the issues are due to the type of exercise training, baseline protein consumption and timing of protein intake. Furthermore, the results are confounded by the fact that some studies include frail adults, while others include only healthy adults. Despite the publication of two systematic reviews, controversy remains as conflicting conclusions emerged from both. Ten Haaf et al. (5) concluded that protein supplementation in older non-frail adults does not lead to increases in LBM or muscle strength compared to control conditions and that it does not exert superior effects when added to resistance exercise training. On the contrary, Morton et al. (3) concluded that dietary protein supplementation in younger and older adults significantly enhanced changes in muscle strength and size during resistance exercise training. Consequently, there is a need to re-examine the literature with an effort to define potential sub-groups within the healthy adult population and consider emerging issues such as the timing of protein intake.

The importance of the timing of protein intake has increased in recent years (6, 7). The concept is to promote a re-distribution of protein intake from mainly during one meal per day to be evenly distributed throughout the day (8). However, the long-term studies examining the impact of protein intake timing have reported conflicting results. No significant differences in skeletal muscle mass or muscle strength were reported with protein supplementation before or after training (9). In a separate study, supplementation immediately after exercise was reported to have an impact on skeletal muscle mass compared to supplementation two hours post-

exercise (10). Furthermore, in adult men and women the within-day distribution of protein did not impact the effect of resistance training on body composition (11). In pre-conditioned older women, the timing of protein intake had no impact on muscle-strength or muscle mass (12). The lack of consistent evidence about the protein supplementation requires further attention to develop an evidence base for dietary recommendations. Consequently, the objective of the present systematic review and meta-analysis was to examine the effect of protein supplementation in healthy adults on body composition and muscle function with a special emphasis on the timing of protein intake. For this purpose, sub-groups within the adult population were examined by age (adults and older adults), body mass index (BMI) (obese group) and timing of protein intake.

Methods

This systematic review and meta-analysis was conducted in accordance to the Cochrane Handbook for Systematic Reviews of Intervention (13) and aimed to answer the following research questions: 1) Does increased protein intake affect body composition and muscle function in primarily healthy adults? and 2) Does the timing of protein intake influence this association? For the purpose of this systematic review, muscle function was defined as muscle strength and muscle synthesis and the timing of protein intake as the timing when the extra amount of protein was consumed, while protein supplementation refers to the higher protein intake achieved with supplements or natural foods. The systematic review protocol is registered on PROSPERO as CRD42019126742.

Search strategy

A systematic search of published data was conducted in Pubmed, Web of Science, CINAHL and Embase to identify all relevant articles published up to the 13th of March 2019. Search terms, keywords, and medical subject headings (MeSH terms, CINAHL headings, or Emtree) according to the PICO acronym were used to define population (adult men and women), intervention (protein intake, protein supplementation), comparison (low-protein diet, no protein supplementation or non-protein placebo - omitted in the search strategy; and timings of protein intake) and outcome (body composition, muscle strength and muscle synthesis) with adaptation for each database where necessary (**Supplementary Table 1**). Furthermore, established filters were used to limit the search results to clinical trials (PubMed filter from Cochrane - Max Sensitivity, EMBASE.com filter from Cochrane, CINAHL filter from SIGN, and Web of Science filter from University of Alberta) (14).

Additionally, relevant articles were identified through reference lists and citations to the included studies. Furthermore, the following clinical trial registry platforms were reviewed for grey literature: ClinicalTrials.gov, the WHO International Clinical Trials Registry Platform, and Lenus.ie.

Study selection and eligibility criteria

Following the removal of duplicate articles, two reviewers (JW and LB) independently screened titles and abstracts for eligibility using the inclusion and exclusion criteria listed in **Table 1**. Disagreements were discussed by the reviewers and resolved through consensus.

In this systematic review, we considered randomized clinical trials of at least two weeks duration that reported the effect of oral protein supplementation or high-protein diet on markers of body composition, muscle strength and/or muscle synthesis. Furthermore, participants had to be free-living and not suffer from any major chronic diseases, such as cancers,

cardiovascular diseases, kidney diseases, diabetes mellitus, chronic obstructive pulmonary disease, systemic sclerosis, HIV, amongst others. However, participants were accepted if they had pre-conditions and risk factors (e.g. metabolic syndrome, hypertension, hyperlipidemia and obesity), as well as disorders related to muscle function, such as frailty and sarcopenia. Subsequently, full texts were retrieved for potentially relevant articles and evaluated for eligibility by two reviewers (EH and JW) using the same inclusion and exclusion criteria as mentioned above (**Table 1**).

Data extraction and synthesis

Data were extracted by two reviewers (EH and JW). Relevant variables included participants characteristics (number, age, sex and BMI), intervention details (group, protein type and amount of intake/supplement, concomitant exercise or energy restriction, and timing of protein intake) and outcomes (body composition, muscle strength and muscle synthesis). Markers of body composition were defined as LBM, fat-free mass, lean soft tissue, skeletal muscle mass, muscle mass, and fat- and bone-free mass measured with dual-energy X-ray absorptiometry or bioelectrical impedance analysis. Furthermore, studies that measured muscle cross-sectional area (CSA) or muscle thickness (MT) were considered for endpoint body composition. The following strength tests were considered for the outcome muscle strength: handgrip, arm flexion, biceps flexion, preacher curl, elbow flexion, arm extension, shoulder press, military press, triceps pushdown, triceps extension, bench press, chest press, chest fly, lateral pull-down, vertical pull-down, horizontal row, back row, squat, back squat, deadlift, leg press, hamstring curl, hip flexion, leg extension, knee extension, knee flexion, ankle dorsiflexion and calf raise. For muscle synthesis, studies on bulk myofibrillar protein synthesis, mixed muscle protein synthesis and fractional synthesis rate were used.

We accepted the following groups as control groups: low-protein diet (LP), no protein supplementation (NP) and non-protein placebo (PLA). However, if a study included two control groups (LP or PLA, and NP), only data from LP or PLA were included in meta-analyses. If a study included more than one protein group (e.g. whey and soy) or timing of protein intake (e.g. morning and after exercise), both groups entered the analyses and were separately compared to the control group. Studies with two protein groups which differed only in the timing of protein intake were used only for meta-analyses on timing, where possible. If a study had a protein group, a control group and one or more groups including additional ingredients (e.g. creatinine, leucine), only the protein and control groups information were included in the meta-analyses. Similarly, we used only information from protein and control groups that did not differ in exercise intervention (e.g. NP with exercise and protein with exercise, but not NP without exercise). Furthermore, in the case of multiple measurements during the intervention, only the ones before and after full intervention length were retrieved and used for meta-analyses.

Meta-analysis

Meta-analyses were performed to estimate the pooled effect size of protein supplementation on LBM, handgrip strength and leg press strength. Changes in the outcomes (standardized to kg) from baseline until the end of the intervention were used. Where changes were not reported and could not be obtained as the post-intervention mean minus the pre-intervention mean, corresponding authors were contacted to provide data. If authors could not be reached and graphs were available, data were extracted using WebPlotDigitizer version 4.2 (Ankit Rohatgi, Texas, USA, 2019). Changes in standard deviation were calculated and imputed according to the Cochrane Handbook for Systematic Reviews of Interventions (13).

Therefore, data from studies with similar design and outcome measurements were used, resulting in correlation coefficients of 0.96 for LBM, 0.93 for handgrip strength and 0.80 for leg press strength (15-21).

Data were uploaded to RevMan software version 5.3 (The Cochrane Collaboration, Copenhagen, Denmark, 2014). To account for heterogeneity across the studies, a random-effects model with the inverse-variance method was applied. Forest plots show mean differences (MD) and 95% confidence intervals (95% CI) of the change in the outcomes. To test the effect of protein supplementation on the outcomes, subjects were classified according to the age groups (adults and older adults). Analyses in obese were performed separately, as the studies usually involved energy restriction and the goal of increased protein intake was rather the maintenance of LBM and muscle strength than an increase in the measurements. For meta-analysis on the timing of protein intake, both adults and older adults groups were merged and outcomes were assessed using indirect comparison, i.e. protein groups were compared to control groups with subgroups representing different timings of protein intake. Meta-analyses of the timing of protein intake for the obese group was performed using head-to-head trials, i.e. studies with two protein groups which differed only in the timing of protein intake.

Risk of bias, heterogeneity and quality of evidence

Risk of bias assessment was conducted according to the Cochrane Handbook for Systematic Reviews of Interventions (13). Only randomized trials were included. Not blinded or single-blinded studies were considered high risk in the domains *allocation concealment* and *blinding of participants and personnel*. Some studies clearly addressed the *blinding of outcome assessment*, but even unclear or open-label studies were judged with low risk since it was assumed that measurements of body composition, muscle strength and muscle synthesis were

not likely to be affected by lack of blinding. A drop-out of 30% or more was considered high risk in the domain *incomplete outcome data*. If data were reported incompletely, this domain was judged with high risk. However, if data could be gathered by the corresponding authors, the judgment changed to low risk. For *other bias*, we considered any conflict of interest and major deviations from the outlined study protocol as high risk. Heterogeneity was determined as the p-value for Chi² test <0.10 and I² test >30%. Funnel plots were visually inspected, and asymmetry was considered suggestive of reporting bias. The quality of the evidence regarding the general outcomes (effect of protein intake on LBM, handgrip strength and leg press strength) was assessed based on meta-analysis and reported according to the GRADE criteria (22), through the use of the GRADEpro software (McMaster University, Hamilton, Canada, 2015), on each of the following domains: risk of bias, inconsistency, imprecision, indirectness and publication bias. The summary of findings for the main comparisons is presented in **Table 2**. All judgments about risk of bias (EH and JW) and quality of evidence (EH and LB) were performed independently by two reviewers and disagreements were resolved through discussions.

Sensitivity and sub-analyses

Sensitivity analyses were conducted for all outcomes by the "remove-one" technique to assess whether individual studies had a disproportionate effect on the results of the meta-analyses. Studies assessed were those with three or more domains judged as high or unclear risk of bias. In order to assess the domain *risk of bias* in the GRADE approach, sensitivity analyses were also conducted with all studies with a risk of bias removed at the same time. Sensitivity analyses were also performed to determine whether the outcomes were dependent

on concomitant exercise intervention (exercise and protein intervention or only protein intervention).

Results

Selection of studies for systematic review and meta-analysis

A total of 2,516 studies from the four databases and 31 studies from additional sources (reference list of included articles or articles that cited included articles) were identified. The search for grey literature did not retrieve relevant completed trials. Following the removal of duplicates and selection of studies according to the eligibility criteria, 65 articles were included in the systematic review and 42 in the meta-analyses (**Supplementary Figure 1**).

Overview of study characteristics

All the included studies ($n = 65$) were randomized clinical trials with parallel groups. Study characteristics are presented for three groups: adults ($n = 30$, **Table 3**), older adults ($n = 23$, **Table 4**) and obese ($n = 12$, **Supplementary Table 2**). Data from 2,907 participants (1514 men and 1380 women) were included in the systematic review. In total, there were 26 studies in men, 15 in women, 23 in men and women, and one did not report the sex ($n = 13$). Mean age and BMI in adults ranged from 20.3 to 52.0 years and 20.4 to 30.4 $\text{kg} \cdot \text{m}^{-2}$, respectively. In this group, 14 studies included subjects who were previously trained in the subsequent type of exercise intervention. The older population was defined by mean age over 55 years, and the mean BMI ranged from 22.9 to 31.3 $\text{kg} \cdot \text{m}^{-2}$. Among these studies, only one (23) included trained subjects. Four studies were conducted with post-menopausal women (24-27) and two

with frail elderly (28, 29). Studies in obese over the age of 55 were included in the obese analysis only. From the twelve studies in obese participants, mean age and BMI ranged from 27.7 to 71 years and 30.9 to 36.5 kg · m⁻², respectively. None of the studies conducted in the obese population reported the inclusion of trained subjects.

Protein supplementation

The number of groups per study ranged from two (n = 41, mostly protein supplement vs. placebo-controlled and high vs. low protein intake) to five (n = 3) with intervention periods from four weeks to two years. Whey, alone or in combination with other proteins or nutrients, was the most often used protein source for supplementation (n = 39). Ten studies reported the use of casein or casein mixtures, while another ten studies used other types of milk proteins. Protein interventions also included soy protein (n = 6), egg white protein (n = 1), beef protein (n = 1), mixed protein shakes (n = 2), protein-enriched foods (n = 5) and natural foods (n = 13). One study did not report the protein source, which was consumed in combination with milk. When protein was provided through supplements, the number of servings per day ranged from one to four and the total supplemented protein amount from 10 g to 106 g per day. Four studies provided dietary counseling for high protein intake without using supplements or enriched foods, for which the number of meals per day ranged from three to six and the total protein intake per day reached up to 142 g.

The timing of protein intake was related to the exercise training in most of the studies: 13 studies supplemented protein immediately after exercise, two before exercise, 11 before and after exercise, eight after exercise plus other time during the day, and one before exercise plus other timing. Furthermore, two studies compared supplementation before exercise vs. after exercise, and two studies compared supplementation after exercise vs. other timing. Two studies with exercise training compared even vs. skewed protein distributions. On non-exercise

days, protein supplementation was most often provided at breakfast (n = 6), at breakfast plus other timing (n = 5), and during the morning (n = 5). Seven studies reported no supplementation on non-exercise days, while 13 were not clear if supplementation was provided. In studies without exercise training, the protein supplementation was most often consumed at breakfast alone (n = 2) or together with one (n = 6) or two (n = 2) other timings. More information on the timing of protein intake is presented in the tables describing the characteristics of the included studies (**Tables 3, Table 4 and Supplementary Table 2**).

Concomitant interventions to protein supplementation

Concomitant interventions to protein supplementation included exercise training and energy restriction. Resistance training alone (22 studies in adults, 13 in older adults and 3 in obese) or combined with other types of exercise (aerobic training n = 3, agility training n = 1, high-intensity interval training n = 1, and endurance training n = 1) was the most frequent concomitant intervention to the protein supplementation. From the 12 studies in obese, eight included energy restriction and five included exercise as part of the intervention protocol. More information on concomitant interventions to protein supplementation is presented in the tables describing the characteristics of the included studies (**Table 3, Table 4 and Supplementary Table 2**).

Impact of protein intake on body composition

Lean body mass

A total of 37 studies investigated the effect of protein supplementation on LBM (**Supplementary Table 3**). In meta-analysis of 26 studies with 31 comparisons, there is moderate quality evidence that protein supplementation improved gains in LBM compared to

control group in both adults (MD: 0.62 kg, 95% CI: 0.36 to 0.88 kg, $p < 0.00001$, $I^2 = 0\%$) (Figure 1A) and older adults (MD: 0.46 kg, 95% CI: 0.23 to 0.70 kg, $p = 0.0001$, $I^2 = 0\%$) (Figure 1B). Considering the meta-analyses of four studies in obese adults, there was no effect of protein supplementation for LBM (MD: 0.04 kg, 95% CI: -0.48 to 0.55 kg, $p = 0.89$, $I^2 = 40\%$) with very low quality evidence (Figure 1C).

Other body composition measurements

Besides LBM, fat-free mass ($n = 4$), lean soft tissue ($n = 3$), muscle mass or skeletal muscle mass ($n = 6$) and fat- and bone-free mass ($n = 3$) were investigated in the included studies. Results were similar to the ones for LBM with most of the protein interventions ($n = 12$) leading to an absolute increase in the assessed parameters, but only a few ($n = 5$) reporting an increase compared to the control groups. Two of three studies with concomitant energy restriction intervention reported a less pronounced decrease in the parameters in protein groups than in controls (Supplementary Table 4).

Cross-sectional area and muscle thickness

CSA and MT were measured in 17 and six studies, respectively, and in a variety of muscles and muscle fibers (Supplementary Table 5). Twelve studies with CSA and four with MT reported an increase for some parameter in the protein group following the intervention, but only three studies (CSA = 2, MT = 1) had a significant increase compared to the control groups.

Impact of protein intake on muscle strength

Handgrip strength

In the meta-analysis of eight studies with older adults, no difference was observed in the effect of protein supplementation on handgrip strength compared to the control condition (MD: 0.26 kg, 95% CI: -0.51 to 1.04 kg, $p = 0.51$, $I^2 = 56\%$) with a moderate quality evidence (**Figure 2**). Only one study investigated the handgrip strength in obese subjects, with a similar increase in protein and control groups. There was no study in younger adults that investigated handgrip strength (**Supplementary Table 6**).

Leg press strength

In the meta-analysis of 13 studies with 16 comparisons, leg press strength in adults showed a tendency to increase with protein supplementation, however, it did not reach statistical significance (MD: 5.80 kg, 95% CI: -0.33 to 11.93 kg, $p = 0.06$, $I^2 = 39\%$) (**Figure 3A**) and the quality of evidence was graded as very low. From meta-analyses with nine studies, there is a moderate quality evidence that protein supplementation in older adults was not effective in improving leg press strength (MD: 1.97 kg, 95% CI: -2.78 to 6.72 kg, $p = 0.42$, $I^2 = 0\%$) (**Figure 3B**). No meta-analysis on leg press strength was conducted within obese adults since data were available from only one study, which observed a similar increase in the leg press strength for both protein and control groups (**Supplementary Table 7**).

Other strength tests

Other strength tests were performed in the selected studies (**Supplementary Table 8**). For simplicity reasons, we only report the results on strength tests that were examined in five or more studies, such as i) bench press ($n = 24$); ii) chest press and chest fly ($n = 7$); iii) lateral and vertical pulldown ($n = 7$); iv) squat, back squat, and deadlift ($n = 10$); v) leg and knee extension ($n = 25$); and vi) leg and knee flexion ($n = 8$). There was no clear consensus on the effect of protein supplementation for these other strength tests.

Impact of timing of protein intake on body composition and muscle strength

Lean body mass

Given the variety of timings of protein supplementation, three groups have been generated for the meta-analysis: after exercise, before and after exercise, and other timing not around exercise. Furthermore, results were pooled for adults and older adults because of similar findings. Protein supplementation improved LBM in all subgroups (after exercise MD: 0.51 kg, 95% CI: 0.13 to 0.89 kg, $p = 0.008$, $I^2 = 1\%$; before and after exercise MD: 0.70 kg, 95% CI: 0.28 to 1.13 kg, $p = 0.001$, $I^2 = 0\%$; and other timing not around exercise MD: 0.52 kg, 95% CI: 0.22 to 0.82 kg, $p = 0.0006$, $I^2 = 17\%$), with no significant difference between the three timings ($p = 0.76$) (**Figure 4**). In meta-analysis comparing even vs. skewed protein supplementation, no pattern presented a superior effect to the other (MD: -0.29 kg; 95% CI: -1.20 to 0.62 kg, $p = 0.62$, $I^2 = 15\%$) (**Figure 5**).

Handgrip strength

For the meta-analysis, data were classified into two subgroups since most of the studies supplemented protein with at least one serving at breakfast. No difference ($p = 0.90$) was observed between protein supplementation at breakfast (MD: 0.44 kg, 95% CI: -1.46 to 2.33 kg, $p = 0.65$, $I^2 = 72\%$) and at breakfast plus another timing during the day (MD: 0.30 kg, 95% CI: -0.81 to 1.40 kg, $p = 0.60$, $I^2 = 54\%$) (**Figure 6**).

Leg press strength

As for LBM, meta-analysis was performed for adults and older adults together and with the three different timing subgroups: after exercise (MD: 10.92 kg, 95% CI: -7.09 to 28.92 kg,

$p = 0.23$, $I^2 = 53\%$), before and after exercise (MD: 4.33 kg, 95% CI: -5.52 to 14.18 kg, $p = 0.39$, $I^2 = 35\%$), and other timing not around exercise (MD: 1.95 kg, 95% CI: -3.06 to 6.96 kg, $p = 0.45$, $I^2 = 0\%$) (**Figure 7**). None of the timings of protein intake had an impact on leg press strength.

Impact of protein intake and timing on muscle protein synthesis

Four studies investigated the impact of protein supplementation on muscle synthesis. Among these, two studies compared the protein supplementation with a control condition. Brodsky et al. (30) observed an 81% lower fractional synthesis rate of myosin heavy chain proteins in vastus lateralis muscle of adults following four weeks of a diet providing $0.71 \text{ g} \cdot \text{kg}^{-1}$ fat-free mass per day compared to a diet providing $1.67 \text{ g} \cdot \text{kg}^{-1}$ fat-free mass per day, with a random protein distribution. In contrast, Robinson et al. (31) did not observe significant differences in mixed muscle protein synthesis rates in middle-aged men and women following six weeks of post-exercise supplementation with 20 g of protein compared to carbohydrate. The other two studies compared the impact of even vs. skewed protein intake on muscle synthesis with the same amount of protein supplemented in both study groups. Kim et al. (32) measured net protein balance, protein synthesis, protein breakdown above basal states, and muscle protein fractional synthesis rates in older men and women, but did not observe differences between groups. Similar findings were reported by Murphy et al. (33) in overweight and obese men who underwent two weeks of energy restriction followed by two weeks of energy restriction combined with resistance exercise and did not present differences in the bulk myofibrillar synthesis rate, as well as fractional synthesis rates of 190 skeletal muscle proteins. Meta-analysis on muscle synthesis was not performed due to the diverse populations and methods for muscle synthesis measurement of the included studies.

Publication bias and sensitivity analyses

Seven trials were completely free of bias (25, 27, 34-38) (**Supplementary Figure 2**). Twenty-three studies presented three or more criteria with an unclear or high risk of bias and, whether data were available for meta-analysis, they were excluded in sensitivity analyses (LBM $n = 8$ (19, 39-46), leg press strength $n = 4$ (39-42), no study for handgrip strength). Visual inspection of funnel plots (**Supplementary Figure 3** and **Supplementary Figure 4**) revealed an asymmetry for leg press strength in adults and older adults with a lack of small studies with null or negative results (left bottom of the graph), which suggests a publication bias for this outcome. Symmetrical funnel plots from other outcomes indicate unlikely publication bias.

The “remove-one” analysis revealed that no single study with potential bias significantly altered the magnitude and direction of the protein supplementation effect on the outcomes. Considering the studies included in meta-analyses, all of those performed with adults had a concomitant exercise training to the protein supplementation, but not those performed with older adults. The removal of studies without exercise training had no impact on analyses. Subjects who did not perform exercise tended to have a positive effect of protein supplementation on LBM and handgrip strength, but LBM was not significant following the removal of a study with potential bias (45) (**Supplementary Table 9**).

Discussion

The present systematic review and meta-analysis was conducted to summarise the impact of protein supplementation on markers of body composition, muscle strength and

muscle synthesis. Furthermore, we evaluated the timing of protein intake as a margin of opportunity for improved prevention measures. Protein supplementation had a significant impact on LBM in adults and older adults, however, the timing of intake did not influence the results. For muscle strength, no significant improvement was observed following the protein supplementation, even though the leg press strength in adults presented a tendency towards improvement. Only a few studies investigated the impact of protein supplementation on muscle synthesis with inconclusive results. Overall, the results support the positive impact of protein supplementation on LBM, independently of the age group and its timing of intake.

Previous meta-analyses have reported inconsistent results regarding the impact of protein supplementation on LBM, with some reporting a positive effect (3, 47, 48) and another concluding that protein supplementation is not effective in improving LBM in non-frail older adults (5). Interestingly, our results demonstrate that protein supplementation has a positive effect on LBM in both adult and older adult groups, even though some studies included frail older adults. In a recent review of 49 studies, Morton et al. (3) concluded that protein supplementation is more effective in improving fat-free mass in younger or resistance-trained individuals than in older or untrained individuals. Our results support the concept that protein supplementation can have an important impact on older adults' health condition. They add to the emerging evidence base for the development of clear protein intake guidelines for improvement and maintenance of LBM with age.

It is also noteworthy that the improvements in LBM were not influenced by the timing of protein intake. While a study suggested the potential importance of an evenly distributed protein intake throughout the day (7), there is no conclusive evidence in the literature (32, 33). Other studies have indicated that pre-sleep protein can support muscle gains during prolonged resistance training (49). Our results corroborate those from Schoenfeld *et al.* (50) who performed a meta-regression and concluded that the immediate protein supplementation pre-

or post-exercise did not improve hypertrophy and muscle strength in adults and older adults. The lack of impact of the timing of protein intake in the present study indicates that the important aspect is to increase the overall protein intake as opposed to altering the distribution throughout the day.

Although there was no overall impact of protein supplementation on the obese group, it has to be acknowledged that this group was very heterogeneous in terms of study design. Among the studies included in the meta-analysis, two had only protein supplementation, one had protein supplementation with energy restriction and one had protein supplementation with exercise intervention. Further work is warranted to investigate if protein supplementation could play a role in preventing the decrease of LBM during weight loss in obese subjects.

In older adults, no beneficial effects of protein supplementation on muscle strength were observed. Our results are similar to those of Hanach *et al.* (51), who did not find an improvement in handgrip strength and leg press strength in older adults with or without sarcopenia following supplementation with dairy protein. Although protein type was not an inclusion criterion, all studies included in our meta-analysis with older subjects mainly contained dairy protein. Dairy products are good sources of high-quality protein, but it is still unclear whether a specific formulation can have better effects than others. Our meta-analysis mainly included non-frail subjects. Recent meta-analyses have suggested a positive effect of protein supplementation on muscle strength for frail (52) but not for non-frail older subjects (5). Hou *et al.* (53) also included both types of participants but found a positive effect of protein supplementation on muscle strength, which could be attributed to their more diverse study designs (energy restriction and supplements not predominantly composed of protein).

Also, in the younger adult population, protein supplementation did not affect leg press strength. The impact of protein supplementation on strength for younger subjects assessed by one-repetition maximum (1-RM) has been controversial across several reviews (3, 48, 50).

Morton et al. (3) found a significant increase in muscle strength in a mixed group of younger and older adults. However, this result might have been influenced by a subgroup of trained subjects, since a null effect of protein supplementation was observed in the untrained subgroup. Besides that, the review pooled studies with different measures of 1-RM. Like Cermak *et al.* (48), we used only leg press to assess muscle strength in order to minimize bias caused by intervention responses in different muscle groups. However, in contrast to our results, they observed significant gains in strength among younger and older adults. Compared to Cermak *et al.* (48), the present analysis was updated to include more studies and has few studies in common (36, 54-56) with the analysis previously published. Further, the previous review included only studies with a prolonged resistance exercise training, while our review also included studies with resistance training but most of them had a shorter period of intervention (<12 weeks intervention). Finally, groups receiving different protein supplements or protein at different timings were merged in the previous analysis while we presented them separately.

The present analysis has some limitations that are worth noting. First, as the criteria were set to include only studies that reported the timing of protein intake, the included studies present important differences in their designs, such as other concomitant interventions, duration of follow-up, training status of participants and types and doses of protein supplementation. However, except for handgrip analysis, such differences were not sufficient to cause high heterogeneity in the meta-analysis. Second, studies that specifically address the timing of protein intake are scarce, and so, except for the comparison in obese (even vs. skewed protein intake), all other analyses encompassing the timing of protein intake were performed by indirect comparison, which must allow less certainty in conclusions than findings from a meta-analysis with head-to-head trials. Nevertheless, our analyses for LBM and leg press strength met the basic assumptions (similarity, homogeneity, and consistency) for the performance of

indirect comparisons (57). Third, we included studies independently of the compliance to the protein supplementation since the majority did not report it.

Strengths of our study include the application of restrictive criteria to the outcomes in the meta-analysis (body composition assessed only by LBM and muscle strength assessed by 1-RM leg press and handgrip) in order to reduce potential variability. Furthermore, we employed the GRADE tool to assess the quality of evidence. Although reviews have been conducted on this topic, our results demonstrate that leg press strength for adults still has low quality evidence to support a final statement regarding the association with protein supplementation.

Conclusions

Overall, the results support the positive impact of protein supplementation on LBM, independently of the age group and its timing of intake. Effects on muscle strength and synthesis are less clear and need further investigation.

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FIGURES

FIGURE 1 Forest plots showing the effect of protein supplementation vs. control on total lean body mass in adults ($n = 512$, range of mean age 19.6-42 y) (A), older adults ($n = 696$, range of mean age 55-81 y) (B) and obese adults ($n = 216$, range of mean BMI 30-50 $\text{kg} \cdot \text{m}^{-2}$) (C). Abbreviations: Chi^2 , chi-squared test; CI, confidence interval; df, degrees of freedom; EX, exercise; I^2 , Higgins's inconsistency statistic; IV, inverse variance; SD, standard deviation; Tau^2 , estimate of between-study variance.

FIGURE 2 Forest plot showing the effect of protein supplementation vs. control on handgrip strength in older adults ($n = 628$, range of mean age 67-81 y). Abbreviations: Chi^2 , chi-squared test; CI, confidence interval; df, degrees of freedom; I^2 , Higgins's inconsistency statistic; IV, inverse variance; SD, standard deviation; Tau^2 , estimate of between-study variance.

FIGURE 3 Forest plot showing the effect of protein supplementation vs. control on leg press strength in adults ($n = 496$, range of mean age 18-42 y) (A) and older adults ($n = 395$, range of mean age 56.1-81 y) (B). Abbreviations: Chi^2 , chi-squared test; CI, confidence interval; df, degrees of freedom; I^2 , Higgins's inconsistency statistic; IV, inverse variance; SD, standard deviation; Tau^2 , estimate of between-study variance.

FIGURE 4 Forest plot of subgroup analysis for the effect of the timing of protein supplementation on total lean body mass in adults and older adults (after exercise = 291, range

of mean age 20.5-68.1 y; before exercise and after exercise = 127, range of mean age 19.6-72 y; not around exercise = 492, range of mean age 19.6-81 y). *Abbreviations:* χ^2 , chi-squared test; CI, confidence interval; df, degrees of freedom; EX, exercise; I^2 , Higgins's inconsistency statistic; IV, inverse variance; SD, standard deviation; τ^2 , estimate of between-study variance.

FIGURE 5 Forest plot showing the effect of even vs. skewed intake of protein on total lean body mass in obese adults ($n = 104$, range of mean BMI 27.2-33.5 $\text{kg} \cdot \text{m}^{-2}$). *Abbreviations:* χ^2 , chi-squared test; CI, confidence interval; df, degrees of freedom; I^2 , Higgins's inconsistency statistic; IV, inverse variance; SD, standard deviation; τ^2 , estimate of between-study variance.

FIGURE 6 Forest plot of subgroup analysis for the effect of timing of protein supplementation on handgrip strength in older adults (at breakfast = 273, range of mean age 69-74.3 y; at breakfast plus other timing = 277, range of mean age 67-81 y). *Abbreviations:* χ^2 , chi-squared test; CI, confidence interval; df, degrees of freedom; I^2 , Higgins's inconsistency statistic; IV, inverse variance; SD, standard deviation; τ^2 , estimate of between-study variance.

FIGURE 7 Forest plot of subgroup analysis for the effect of timing of protein supplementation on leg press strength in adults and older adults (after exercise = 107, range of mean age 20.5-68.7 y; before exercise and after exercise = 165, range of mean age 20-72 y; not around exercise = 344, range of mean age 21-81 y). *Abbreviations:* χ^2 , chi-squared test; CI, confidence

interval; df, degrees of freedom; EX, exercise; I^2 , Higgins's inconsistency statistic; IV, inverse variance; SD, standard deviation; Tau^2 , estimate of between-study variance.

TABLE 1 Inclusion and exclusion criteria applied to studies investigating the effect of protein intake on body composition in healthy adults

| | Inclusion criteria | Exclusion criteria |
|--------------|---|---|
| Population | Free-living Adults Healthy people or people with a high risk of chronic diseases (metabolic syndrome, hypertension, impaired glucose, high blood lipids, overweight, and obesity) Frail or sarcopenic people | Hospitalized or immobilized Assisted-living situation Participants with diseases or conditions affecting muscle function (frailty, sarcopenia, etc.) Children Animal studies |
| Intervention | Randomized controlled trials with oral protein intake 2 weeks minimal duration Including energy-restriction or not Including exercise or not | Only dietary advice or behavioral intervention Non-oral feeding No timing of protein intake Essential amino acids alone Intervention group with total energy intake < 25% of total energy intake or 25% of total energy intake lower than 10 g · d ⁻¹ Protein intake only on single day |
| Comparison | Groups differ in the timing of protein intake Groups differ in the protein amount and the timing of protein intake is mentioned | Comparison of two protein treatments Difference between the groups is not mentioned Intervention or ingredient (e.g., protein, amino acids, micronutrients) Methods other than dual-energy X-ray absorptiometry, bioelectrical impedance analysis, or other validated methods |
| Outcome | Body composition Muscle strength Muscle synthesis | Methods other than dual-energy X-ray absorptiometry, bioelectrical impedance analysis, or other validated methods |
| Others | English and German language | Observational studies, methodological reviews, letters to the editor, conference abstracts |

TABLE 2 Summary of findings from the studies investigating the effect of protein intake on body composition in adults ¹

| Outcomes | Anticipated absolute effects ² Increased risk with protein intake. kg MD (95% CI) | Nº of participants (studies) |
|------------------------------------|---|-------------------------------------|
| LBM in adults | 0.62 (0.36, 0.88) | 512 (13 RCTs) |
| LBM in older adults | 0.46 (0.23, 0.70) | 696 (13 RCTs) |
| LBM in obese | 0.04 (-0.48, 0.55) | 216 (4 RCTs) |
| Handgrip strength in older adults | 0.26 (-0.51, 1.04) | 628 (8 RCTs) |
| Leg press strength in adults | 5.8 (-0.33, 11.93) | 496 (13 RCTs) |
| Leg press strength in older adults | 1.97 (-2.78, 6.72) | 395 (9 RCTs) |

¹ The findings refer to free-living adults and older adults who underwent an intervention with high diet protein and were compared to a control or placebo group. CI, confidence interval; LBM, lean body mass; MD, mean difference; RCT, randomised controlled trials.

² The risk in the intervention group (and its 95% confidence interval) is based on the assumed risk in the comparison of no intervention or the effect of the intervention (and its 95% CI).

³ HIGH certainty: We are very confident that the true effect lies close to that of the estimate of the effect. MODERATE certainty: We are moderately confident in the effect estimate: The true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different. LOW certainty: Our confidence in the effect estimate is limited: The true effect may be close to the estimate of the effect, but it is likely that it is different. VERY LOW certainty: We have very little confidence in the effect estimate: The true effect is likely to be different from the estimate of effect

⁴ We downgraded the evidence by one level because, despite the risk of bias, the effect size was not significant. Studies presented unclear or high risk of bias considering allocation concealment (n = 12), blinding of participants (n = 5), potential funding conflict (n = 5), and high drop-out rate (n = 4).

⁵ We downgraded the evidence by one level because, despite the risk of bias, the effect size was not significant. Studies presented unclear or high risk of bias considering allocation concealment (n = 10), blinding of participants (n = 3), potential funding conflict (n = 3), and high drop-out rate (n = 1).

⁶ We downgraded the evidence by one level because, despite the risk of bias, the effect size was not significant. Studies presented unclear or high risk of bias considering allocation concealment (n = 3), blinding of participants (n = 3), potential funding conflict (n = 3), and selective reporting (n = 1).

⁷ We downgraded the evidence by one level due to the moderate heterogeneity verified by $\text{Chi}^2 > \text{degrees of freedom}$.

⁸ We downgraded the evidence by two levels due to the wide confidence interval and total sample size <400. = We did not downgrade the evidence since the risk of bias was not so relevant and the effect size was not significant in the meta-analyses. Studies presented unclear or high risk of bias considering allocation concealment (n = 6), blinding of participants (n = 2), and potential funding conflict (n = 1).

¹⁰ We downgraded the evidence by one level because, despite the risk of bias, the effect size was not significant. Studies presented unclear or high risk of bias considering allocation concealment (n = 10), blinding of participants (n = 3), drop-out rate (n = 3), potential funding conflict (n = 2), and selective reporting (n = 1).

¹¹ Suspected publication bias according to the visual inspection of funnel plot.

TABLE 3 Characteristics of studies investigating the effect of protein intake on body composition and muscle strength in adults ¹

| Study | Group | n (M, F) | Age, y ² | BMI, kg · m ^{-2.2} | Physical activity status | Exercise intervention | Duration, wk | Protein type | Servings, n/d | Supp |
|--------------------|----------|-------------|-------------------------|-----------------------------|--------------------------|----------------------------|--------------|--------------------|---------------|--------------------------|
| | | | | | | | | | | Protein added, g/ |
| Arciero 2016 (39) | P | 12 (0, 12) | 42 (9) | 24 (2) | T | RISE 4x per wk | 12 | W bars | 5 - 6 | NR |
| | CON | 15 (0, 15) | 42 (7) | 24 (3) | T | RISE 4x per wk | 12 | - | 5 - 6 | NR |
| Brodsky 2004 (30) | HP | 6 (4, 2) | 25.5 ± 1.4 | 23.5 ³ | NR | - | 4 | AV food sources | 6 | - |
| Burke 2001 (58) | LP | 8 (4, 4) | 25.3 ± 1.8 | 24.0 ² | NR | - | 4 | - | 6 | - |
| | P1 | 6 (6, 0) | 18-31 ⁴ | NR | T | RT 4x per wk | 6 | W | 4 | 1.2 g · kg ⁻¹ |
| | P2 | 7 (7, 0) | 18-31 ⁴ | NR | T | RT 4x per wk | 6 | W + Cr | 4 | 1.3 g · kg ⁻¹ |
| Campbell 2018 (59) | CON | 6 (6, 0) | 18-31 ⁴ | NR | T | RT 4x per wk | 6 | - | 4 | - |
| | P1 | 8 (0, 8) | 21.5 (2.3) ⁶ | 22.0 (1.8) ⁶ | T | RT 4x per wk | 8 | W | 2 | 50 |
| | P1 | 9 (0, 9) | 20.9 (2.0) ⁶ | 22.9 (1.8) ⁶ | T | RT 4x per wk | 8 | W | 2 | 10 |
| Coburn 2006 (60) | P | 11 (11, 0) | 21.3 (2.0) | 23.5 ³ | UT | RT 3x per wk | 8 | W + Leu | 1 - 2 | 26.2-52.4 |
| | CON | 12 (12, 0) | 22.8 (2.8) | 25.1 ³ | UT | RT 3x per wk | 8 | - | 1 - 2 | - |
| | NP | 10 (10, 0) | 23.2 (1.9) | 25.3 ³ | UT | - | 8 | - | - | - |
| Hartman 2007 (54) | P1 | 18 (18, 0) | 18-30 ⁴ | 25.1 ± 1.2 | UT | RT 5x per wk | 12 | Fat-free milk | 2 | 35 |
| | P2 | 19 (19, 0) | 18-30 ⁴ | 26.0 ± 1.4 | UT | RT 5x per wk | 12 | Fat-free soy drink | 2 | 35 |
| | CON | 19 (19, 0) | 18-30 ⁴ | 24.6 ± 1.2 | UT | RT 5x per wk | 12 | - | 2 | - |
| Herda 2013 (61) | P1 | 22 (22, 0) | 21.1 (2.5) | 25.0 ³ | T | RT 3x per wk - low volume | 8 | bW | 1 - 2 | 20-40 |
| | P1 | 20 (20, 0) | 21.2 (2.7) | 25.0 ³ | T | RT 3x per wk | 8 | bW | 1 - 2 | 20-40 |
| | P2 | 22 (22, 0) | 21.0 (1.6) | 23.6 ³ | T | RT 3x per wk | 8 | W | 1 - 2 | 20-40 |
| Hida 2012 (62) | CON | 21 (21, 0) | 20.9 (1.7) | 24.6 ³ | T | RT 3x per wk | 8 | - | 1 - 2 | - |
| | NP | 21 (21, 0) | 21.1 (2.4) | 25.0 ³ | T | RT 3x per wk | 8 | - | - | - |
| | P | 15 (0, 15) | 18-22 ⁴ | 22.2 ^{3,4} | T | TS ≥6x per wk ⁷ | 8 | EW | 1 | 15 |
| Hoffman 2009 (19) | CON | 15 (0, 15) | 18-22 ⁴ | 22.2 ^{3,4} | T | TS ≥6x per wk ⁷ | 8 | - | 1 | - |
| | P1 | 13 (13, 0) | 19.6 (1.3) | 30.4 ³ | T | RT 4x per wk | 10 | CG + W + C | 2 | 42 |
| | P1 | 13 (13, 0) | 19.9 (1.3) | 28.3 ³ | T | RT 4x per wk | 10 | CG + W + C | 2 | 42 |
| Huang 2017 (63) | NP | 7 (7, 0) | 20.7 (1.1) | 31.1 ³ | T | RT 4x per wk | 10 | - | - | - |
| | P | 6 (6, 0) | 21.7 (2.7) | 20.6 (0.7) | T | AT 7x per wk ⁷ | 5 | W | 1 | 33.5 |
| | CON | 6 (6, 0) | 21.0 (2.0) | 20.4 (0.9) | T | AT 7x per wk ⁷ | 5 | - | 1 | - |
| Hulmi 2009 (36) | P | 11 (11, 0) | 25.2 (5.2) | 23.0 ³ | UT | RT 2x per wk | 21 | W | 2 | 30 |
| | CON | 10 (10, 0) | 27.2 (3.0) | 22.8 ³ | UT | RT 2x per wk | 21 | - | 2 | - |
| | NP | 10 (10, 0) | 24.9 (2.7) | 22.7 ³ | UT | - | 21 | - | - | - |
| Hwang 2017 (64) | P | 11 (11, 0) | 20.9 (1.4) | 24.9 ^{3,4} | T | RT 4x per wk | 10 | W | 1 | 25 |
| | CON | 9 (9, 0) | 21.0 (1.1) | 24.9 ^{3,4} | T | RT 4x per wk | 10 | - | 1 | - |
| Josse 2010 (55) | P | 10 (0, 10) | 23.2 ± 2.8 | 26.2 ± 4.2 | UT | RT 5x per wk | 12 | Fat-free milk | 2 | 36 |
| | CON | 10 (0, 10) | 22.4 ± 2.4 | 25.2 ± 3.8 | UT | RT 5x per wk | 12 | - | 2 | - |
| Joy 2018 (20) | P1 + CON | 13-x (y, 0) | 18-25 ⁴ | 25.1 ³ | UT | RT 4x per wk | 10 | C | 1 | 35 |
| | P1 + CON | 13-y (x, 0) | 18-25 ⁴ | 24.6 ³ | UT | RT 4x per wk | 10 | C | 1 | 35 |
| Lockwood 2017 (21) | P1 | 13 (13, 0) | 21.3 ± 0.7 | 25.9 ³ | T | RT 4x per wk | 8 | W | 2 | 60 |
| | P2 | 15 (15, 0) | 21.8 ± 0.9 | 24.9 ³ | T | RT 4x per wk | 8 | W + LF | 2 | 60 |
| | P3 | 13 (13, 0) | 21.5 ± 0.9 | 25.1 ³ | T | RT 4x per wk | 8 | Wh | 2 | 60 |

| | | | | | | | | | | |
|----------------------|-----|--------------------------|-------------------------|---------------------|----|------------------------|----|---------------------|---|--------------------------|
| Mobley 2017 (65) | CON | 15 (15, 0) | 20.9 ± 0.4 | 23.8 ³ | T | RT 4x per wk | 8 | - | 2 | - |
| | P1 | 14 (14, 0) | 20 ± 1 | 23.4 ³ | UT | RT 3x per wk | 12 | Leu | 2 | 4.6 |
| | P2 | 17 (17, 0) | 21 ± 1 | 25.3 ³ | UT | RT 3x per wk | 12 | W + Leu | 2 | 52.6 |
| | P3 | 14 (14, 0) | 21 ± 1 | 23.8 ³ | UT | RT 3x per wk | 12 | Wh + Leu | 2 | 50.8 |
| | P4 | 15 (15, 0) | 21 ± 1 | 24.5 ³ | UT | RT 3x per wk | 12 | S + Leu | 2 | 78.4 |
| Naclerio 2017 (66) | CON | 15 (15, 0) | 21 ± 1 | 23.6 ³ | UT | RT 3x per wk | 12 | - | 2 | - |
| | P1 | 8 (8, 0) | 25 (8) | 24.5 ³ | T | RT 3x per wk | 8 | BF | 1 | 16.4 |
| | P2 | 8 (8, 0) | 26 (5) | 24.1 ³ | T | RT 3x per wk | 8 | W | 1 | 18 |
| Negro 2014 (67) | CON | 8 (8, 0) | 29 (9) | 25.2 ³ | T | RT 3x per wk | 8 | - | 1 | - |
| | P | 12 (8, 4) | 23.7 (2.5) | 23.5 (4.7) | UT | RT 3x per wk | 8 | Lean beef | 1 | 20 |
| | NP | 14 (11, 3) | 23.9 (4.2) | 24.1 (3.5) | UT | RT 3x per wk | 8 | - | - | - |
| Ormsbee 2018 (41) | P | 29 (16, 13) ⁶ | 21.0 ± 0.6 | NR | UT | AT + RT 5x per wk | 24 | Mixed protein shake | 2 | 84 |
| | CON | 22 (11, 11) ⁶ | 20.3 ± 0.5 | NR | UT | AT + RT 5x per wk | 24 | - | 2 | - |
| Pihoker 2019 (42) | P1 | 17 (0, 17) | 20.5 (2.2) ⁴ | 24.4 ^{3,4} | T | RT 2x per wk | 6 | Mixed protein shake | 1 | 25 |
| | P1 | 17 (0, 17) | 20.5 (2.2) ⁴ | 24.4 ^{3,4} | T | RT 2x per wk | 6 | Mixed protein shake | 1 | 25 |
| | NP | 9 (0, 9) | 20.5 (2.2) ⁴ | 24.4 ^{3,4} | T | RT 2x per wk | 6 | - | - | - |
| Reidy 2016 (68) | P1 | 23 (23, 0) | 24 ± 1 | 24.4 ± 0.6 | UT | RT 3x per wk | 12 | W + S + C | 1 | 22 |
| | P2 | 22 (22, 0) | 25 ± 1 | 25.8 ± 0.7 | UT | RT 3x per wk | 12 | W | 1 | 22 |
| | CON | 23 (23, 0) | 25 ± 1 | 24.6 ± 0.6 | UT | RT 3x per wk | 12 | - | 1 | - |
| Robinson 2011 (31) | P | 8 (3, 5) | 48 (7) | 24.7 (3.3) | UT | AT 3x per wk | 6 | MP | 1 | 20 |
| | CON | 8 (3, 5) | 52 (10) | 25.3 (4.8) | UT | AT 3x per wk | 6 | - | 1 | - |
| | NP | 4 (1, 3) | 21 (2) | 22.6 (1.9) | UT | - | 6 | - | - | - |
| Rozenek 2002 (56) | P | 26 (26, 0) | 23.0 (4.6) | 24.6 ³ | UT | RT 4x per wk | 8 | NR + milk | 2 | 106 |
| | CON | 25 (25, 0) | 23.4 (4.7) | 23.5 ³ | UT | RT 4x per wk | 8 | Milk | 2 | 24 |
| | NP | 21 (21, 0) | 23.1 (3.9) | 26.4 ³ | UT | RT 4x per wk | 8 | - | - | - |
| Schoenfeld 2017 (69) | P1 | 9 (9, 0) | 22.9 (3.0) ⁴ | 26.9 ^{3,4} | T | RT 3x per wk | 10 | Wh | 1 | 25 |
| | P1 | 12 (12, 0) | 22.9 (3.0) ⁴ | 26.9 ^{3,4} | T | RT 3x per wk | 10 | Wh | 1 | 25 |
| Snijders 2015 (49) | P | 20 (20, 0) | 23 ± 1 | 23.2 ± 0.6 | UT | RT 3x per wk | 12 | Ch + C | 1 | 27.5 |
| | CON | 19 (19, 0) | 21 ± 1 | 23.4 ± 0.8 | UT | RT 3x per wk | 12 | - | 1 | - |
| Taylor 2016 (70) | P | 8 (0, 8) | 20 ± 2 | 22.7 ³ | T | AgT + ET+ RT 4x per wk | 8 | W | 2 | 48 |
| | CON | 6 (0, 6) | 21 ± 3 | 23.7 ³ | T | AgT + ET+ RT 4x per wk | 8 | - | 2 | - |
| Vangsoe 2018 (71) | P | 9 (9, 0) | 24.2 (2.6) ⁴ | 22.9 ^{3,4} | NR | RT 4x per wk | 8 | IP bars | 2 | 0.4 g · kg ⁻¹ |
| | CON | 9 (9, 0) | 24.2 (2.6) ⁴ | 22.9 ^{3,4} | NR | RT 4x per wk | 8 | - | 2 | - |
| Volek 2013 (72) | P1 | 19 (13, 6) | 22.8 (3.7) | 25.1 ³ | NR | RT 3x per wk | 36 | W | 1 | 21.6 |
| | P2 | 22 (11, 11) | 24.0 (2.9) | 24.8 ³ | NR | RT 3x per wk | 36 | S | 1 | 20 |
| | CON | 22 (13, 9) | 22.3 (3.1) | 24.5 ³ | NR | RT 3x per wk | 36 | - | 1 | - |
| Walker 2010 (43) | P | 18 (18, 0) | 26.9 ^{4,9} | NR | T | AT + RT ≥3x per wk | 8 | W + Leu | 2 | 51.8 |
| | CON | 12 (12, 0) | 26.9 ^{4,9} | NR | T | AT + RT ≥3x per wk | 8 | - | 2 | - |
| Weisgarber 2012 (44) | P | 9 (5, 4) | 24.9 (1.8) | 29.7 ³ | UT | RT 4x per wk | 8 | W | 2 | 0.3 g · kg ⁻¹ |
| | CON | 8 (4, 5) | 23.6 (4.4) | 28.8 ³ | UT | RT 4x per wk | 8 | - | 2 | - |

¹ AV, animal and vegetable; AE, after exercise; AgT, agility training; AT, aerobic training; BB, before breakfast; BE, before exercise; BF, before feed; BS, before sleep; bW, bioenhanced whey protein; C, casein; CG, collagen; Ch, hydrolyzed casein; CHO, carbohydrate; CON, control group; ET, endurance training; IP, isometric power; MP, mixed protein; NR, no resistance training; P, post-exercise; P1, post-exercise 1; P2, post-exercise 2; P3, post-exercise 3; P4, post-exercise 4; RT, resistance training; S, soy protein; T, testosterone; UT, untrained; W, whey protein; Wh, whey hydrolysate; Y, yeast; Z, zein.

ET, explosive training; EV, evenly; EW, egg white protein; EX, exercise; F, female; HP, high protein diet; IP, insect protein; Leu, Leucine; L, morning to afternoon; Mo, morning; MP, milk protein; NC, non-caloric; NP, no protein supplemented; NR, not reported; P, protein; R, random; RT, resistance training; S, soy; T, trained; TS, team sports; UT, untrained; W, whey protein; Wh, hydrolyzed whey.

² Data presented as mean (SD), mean \pm SEM, or range.

³ Data calculated as the mean weight (kg) divided by the square of mean height (m²).

⁴ No group information available and overall values used.

⁵ Individualized amount based on body weight.

⁶ Data retrieved after contacting the corresponding author.

⁷ No actual exercise intervention, but participants performed regular training.

⁸ Data calculated from average weight and grams of protein per day.

⁹ No indicator of variation presented.

TABLE 4 Characteristics of studies investigating the effect of protein intake on body composition and muscle strength in older adults ¹

| Study | Group | n (M, F) | Age, y ² | BMI, kg · m ^{-2.2} | Physical activity status | Exercise intervention | Duration, wk | Protein type | Servings, n/d | Protein added, g/d |
|---------------------|--------|-------------|-------------------------|------------------------------|--------------------------|-----------------------------------|--------------|----------------------|---------------|--------------------|
| Bell 2017 (34) | P | 25 (25, 0) | 71 ± 1 | 28.9 ± 0.8 | NR | RT 2x per wk from wk ⁷ | 20 | W + Cr | 2 | 60 |
| | CON | 24 (24, 0) | 74 ± 1 | 28.1 ± 0.7 | NR | RT 2x per wk from wk ⁷ | 20 | - | 2 | - |
| Bemben 2010 (73) | P1 | 11 (11, 0) | 58.2 ± 2.0 | 28.6 ³ | UT | RT 3x per wk | 14 | W | 1 | 35 |
| | P2 | 10 (10, 0) | 56.1 ± 1.8 | 28.9 ³ | UT | RT 3x per wk | 14 | Cr | 1 | 5 |
| | P3 | 11 (11, 0) | 57.2 ± 2.2 | 28.7 ³ | UT | RT 3x per wk | 14 | W + Cr | 1 | 40 |
| | CON | 10 (10, 0) | 56.1 ± 1.4 | 31.3 ³ | UT | RT 3x per wk | 14 | - | 1 | - |
| Chale 2013 (35) | P | 42 (17, 25) | 78.0 (4.0) | 27.0 (3.2) | UT | RT 3x per wk | 24 | W | 2 | 40 |
| | CON | 38 (16, 22) | 77.3 (3.9) | 26.9 (3.1) | UT | RT 3x per wk | 24 | - | 2 | - |
| Chanet 2017 (74) | P | 12 (12, 0) | 70.3 (4.3) | 24.4 (3.3) | UT | - | 6 | W + Leu | 1 | 20 |
| | CON | 12 (12, 0) | 70.8 (3.5) | 25.1 (2.5) | UT | - | 6 | - | 1 | - |
| Daly 2014 (75) | P | 53 (0, 53) | 72.1 (6.4) | 27.7 (3.9) | UT | AgT + RT 2x per wk | 16 | Lean red meat | 2 | 45 |
| | CON | 47 (0, 47) | 73.6 (7.7) | 27.6 (4.8) | UT | AgT + RT 2x per wk | 16 | - | 2 | - |
| de Branco 2019 (24) | P1/CON | 17 (0, 17) | 60.5 [57.7-63.3] | 27.9 [25.2-30.8] | UT | RT 3x per wk | 8 | W | 1 | 30 |
| | P1/CON | 17 (0, 17) | 61.4 [58.0-65.0] | 27.6 [25.7-29.7] | UT | RT 3x per wk | 8 | W | 1 | 30 |
| Esmarck 2001 (10) | P1 | 7 (7, 0) | 74 ± 1 | 26 ± 1 | UT | RT 3x per wk | 12 | MP + S | 1 | 10 |
| | P1 | 6 (6, 0) | 73 ± 1 | 25 ± 1 | UT | RT 3x per wk | 12 | MP + S | 1 | 10 |
| Gryson 2014 (76) | P1 | 9 (9, 0) | 60.9 ± 0.5 ⁵ | 26.8 ± 0.9 | UT | AT + RT 3x per wk | 16 | C + MP | 1 | 10 |
| | P1 | 9 (9, 0) | 60.9 ± 0.5 ⁵ | 26.2 ± 0.6 | UT | AT + RT 3x per wk | 16 | C + MP | 1 | 4 |
| | P1 | 9 (9, 0) | 60.5 ± 0.7 ⁵ | 26.5 ± 0.8 | UT | - | 16 | C + MP | 1 | 4 |
| | P2 | 8 (8, 0) | 60.9 ± 0.5 ⁵ | 26.8 ± 1.2 | UT | AT + RT 3x per wk | 16 | MP + Leu | 1 | 10 |
| | P2 | 10 (10, 0) | 60.5 ± 0.7 ⁵ | 25.9 ± 0.9 | UT | - | 16 | MP + Leu | 1 | 10 |
| Holm 2008 (26) | P | 13 (0, 13) | 55 ± 1 | 24 ± 1 | NR | RT 3x per wk | 24 | W | 1 | 10 |
| | CON | 16 (0, 16) | 55 ± 1 | 27 ± 1 | NR | RT 3x per wk | 24 | - | 1 | - |
| Kim 2018 (32) | P | 7 (4, 3) | 58.1 ± 2.4 | 27.7 ± 0.6 | UT | - | 8 | Egg, dairy, and beef | 3 | - |
| | P | 7 (2, 5) | 60.3 ± 2.4 | 27.2 ± 0.7 | UT | - | 8 | Egg, dairy, and beef | 3 | - |
| Leenders 2013 (16) | P | 27 (15, 12) | M: 70 ± 1, F: 72 ± 2 | M: 27.2 ± 0.7, F: 24.2 ± 0.7 | UT | RT 3x per wk | 24 | W + C | 1 | 15 |
| | CON | 26 (14, 12) | M: 70 ± 1, F: 69 ± 1 | M: 26.7 ± 0.6, F: 25.0 ± 0.4 | UT | RT 3x per wk | 24 | - | 1 | - |
| Nabuco 2018 (12) | P1/CON | 22 (0, 22) | 67.5 (5.2) | 26.4 (5.2) | UT | RT 3x per wk | 12 | Wh | 1 | 27. |

| | | | | | | | | | | |
|----------------------|--------|--------------|---------------------|-------------------|----|----------------|-----|-----------------------|---|-----------|
| | P1/CON | 21 (0, 21) | 66.2 (9.4) | 25.3 (5.4) | UT | RT 3x per wk | 12 | Wh | 1 | 27. |
| Norton 2016 (45) | CON | 23 (0, 23) | 66.5 (7.2) | 23.8 (3.7) | UT | RT 3x per wk | 12 | - | 2 | - |
| | P | 31 (9, 22) | 62.2 (4.7) | 25.7 (3.1) | NR | - | 24 | MP | 2 | 0.3 16 |
| Orsatti 2018 (25) | CON | 29 (5, 24) | 59.5 (5.8) | 25.9 (4.1) | NR | - | 24 | - | 2 | - |
| | P | 16 (0, 16) | 56.8 (6.6) | 27.5 (4.0) | UT | RT 2-3x per wk | 16 | S + fat-free milk | 1 | 31. |
| Ottestad 2017 (77) | CON | 16 (0, 16) | 58.8 (8.9) | 27.3 (4.1) | UT | RT 2-3x per wk | 16 | Fat-free milk | 1 | 6.4 |
| | P | 17 (5, 12) | 76.8 (6.2) | 27.6 (4.2) | NR | - | 12 | Protein enriched milk | 2 | 40. |
| Seino 2018 (78) | CON | 19 (7, 12) | 77.1 (4.7) | 25.9 (4.9) | NR | - | 12 | - | 2 | - |
| | P | 40 (6, 34) | 73.4 (4.3) | 22.9 (2.9) | UT | RT 2x per wk | 12 | Protein enriched milk | 1 | 10. |
| ten Haaf 2019 (23) | NP | 40 (7, 33) | 73.7 (4.3) | 22.9 (2.2) | UT | RT 2x per wk | 12 | - | - | - |
| | P | 58 (47, 11) | 67-72 ⁵ | 27.2 (2.6) | T | AT ?x per wk | 12 | MP | 2 | 31 |
| | CON | 56 (46, 10) | 67-73 ⁵ | 26.3 (2.5) | T | AT ?x per wk | 12 | - | 2 | - |
| Tieland 2012 (28) | P | 34 (14, 20) | 78 ± 1 | 27.0 ± 0.6 | UT | - | 24 | MP | 2 | 30 |
| | CON | 31 (15, 16) | 81 ± 1 | 26.2 ± 0.6 | UT | - | 24 | - | 2 | - |
| Tieland 2012a (29) | P | 31 (11, 20) | 78 (9) | 28.7 (4.5) | UT | RT 2x per wk | 24 | MP | 2 | 30 |
| | CON | 31 (10, 21) | 79 (6) | 28.2 (4.6) | UT | RT 2x per wk | 24 | - | 2 | - |
| Verdijk 2009 (79) | P | 13 (13, 0) | 72 ± 2 ⁵ | 26.5 ± 1.0 | UT | RT 3x per wk | 12 | Ch | 2 | 20 |
| | CON | 13 (13, 0) | 72 ± 2 ⁵ | 27.4 ± 1.1 | UT | RT 3x per wk | 12 | - | 2 | - |
| Villanueva 2014 (80) | P | 7 (7, 0) | 68.7 (6.8) | 25.3 ³ | UT | RT 3x per wk | 12 | W + Cr | 1 | 35 |
| | NP | 7 (7, 0) | 68.7 (6.6) | 27.1 ³ | UT | RT 3x per wk | 12 | - | - | - |
| | NP | 8 (8, 0) | 67.1 (5.9) | 26.8 ³ | UT | - | 12 | - | - | - |
| Vorup 2017 (81) | HP | 13 (6, 7) | 69 (3) | 26 (4) | UT | TS 2x per wk | 12 | Low-fat milk + MP | 2 | 36 |
| | LP | 18 (8, 10) | 74 (6) | 28 (4) | UT | TS 2x per wk | 12 | - | 2 | 6 |
| | NP | 17 (8, 9) | 72 (7) | 25 (5) | UT | - | 12 | - | - | - |
| Zhu 2015 (27) | P | 101 (0, 101) | 74.2 (2.8) | 26.1 (3.8) | NR | - | 104 | W | 1 | 30 |
| | CON | 95 (0, 95) | 74.3 (2.6) | 27.2 (4.0) | NR | - | 104 | - | 1 | - |

¹ A, afternoon; AB, after breakfast; AD, after dinner; AE, after exercise; AgT, agility training; AL, after lunch; AT, aerobic training; B, breakfast; BMI, body mass index; BS, before sleep; C, casein; Ch, hydrolyzed casein; CHO, carbohydrate; CON, control group; Cr, creatine; D, dinner; EV, evening; IC, isocaloric; L, lunch; Leu, Leucine; LP, low protein diet; M, male; Mo, morning; MP, milk protein; NC, non-caloric; NP, no protein supplement; P, protein; S, soy; SK, skewed; T, trained; TS, team sports; UT, untrained; W, whey protein; Wh, hydrolyzed whey.

² Data presented as mean (SD), mean ± SEM, mean [95% confidence interval], or range.

³ Data calculated as the mean weight (kg) divided by the square of mean height (m²).

⁴ Data calculated from average weight and grams of protein per day.

⁵ No group information available and overall values used.

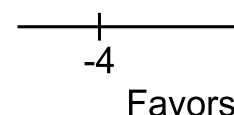
⁶ Individualized amount based on body weight.

| | | |
|----------------------|------|--------------------|
| Walker 2010 (64) | 3.2% | 0.30 [-1.16, 1.76] |
| Weisgarber 2012 (65) | 1.1% | 0.20 [-2.29, 2.69] |

Total (95% CI) **100.0%** **0.62 [0.36, 0.88]**

Heterogeneity: $\tau^2 = 0.00$; $\chi^2 = 10.70$, $df = 16$ ($P = 0.83$); $I^2 = 0\%$

Test for overall effect: $Z = 4.68$ ($P < 0.00001$)



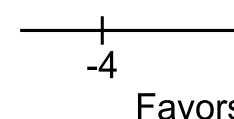
B

| Study or Subgroup | Weight | Mean Difference |
|-----------------------------|--------|--------------------|
| | | IV, Random, 95% CI |
| Bell 2017 (66) | 9.2% | 1.37 [0.60, 2.14] |
| Chale 2013 (68) | 4.9% | 0.30 [-0.75, 1.35] |
| Chanet 2017 (69) | 4.4% | 0.50 [-0.62, 1.62] |
| Daly 2014 (70) | 9.1% | 0.50 [-0.27, 1.27] |
| Holm 2008 (26) | 5.9% | 0.33 [-0.64, 1.29] |
| Leenders 2013 - female (16) | 4.5% | 0.20 [-0.90, 1.30] |
| Leenders 2013 - male (16) | 3.6% | 0.40 [-0.84, 1.64] |
| Norton 2016 (73) | 23.0% | 0.61 [0.12, 1.10] |
| Ottestad 2017 (74) | 18.5% | 0.00 [-0.54, 0.54] |
| ten Haaf 2019 (23) | 6.6% | 0.23 [-0.68, 1.14] |
| Tieland 2012 (28) | 3.1% | 0.10 [-1.23, 1.43] |
| Tieland 2012a (29) | 2.6% | 1.60 [0.15, 3.05] |
| Verdijk 2009 (76) | 3.7% | 0.10 [-1.12, 1.32] |
| Villanueva 2014 (77) | 1.0% | 0.85 [-1.47, 3.17] |

Total (95% CI) **100.0%** **0.46 [0.23, 0.70]**

Heterogeneity: $\tau^2 = 0.00$; $\chi^2 = 12.24$, $df = 13$ ($P = 0.51$); $I^2 = 0\%$

Test for overall effect: $Z = 3.89$ ($P = 0.0001$)



C

| Study or Subgroup | Weight | Mean Difference |
|----------------------------|--------|---------------------|
| | | IV, Random, 95% CI |
| Ormsbee 2015 - casein (31) | 7.7% | -0.40 [-2.08, 1.28] |
| Ormsbee 2015 - whey (31) | 11.0% | -0.30 [-1.64, 1.04] |
| Pal 2010 - casein (17) | 25.5% | -0.40 [-1.06, 0.26] |
| Pal 2010 - whey (17) | 16.0% | -0.44 [-1.46, 0.58] |
| Smith 2018 (33) | 21.4% | 0.40 [-0.39, 1.19] |
| Wright 2018 (18) | 18.4% | 1.01 [0.10, 1.92] |

Total (95% CI) **100.0%** **0.04 [-0.48, 0.55]**

Heterogeneity: $\tau^2 = 0.16$; $\chi^2 = 8.29$, $df = 5$ ($P = 0.14$); $I^2 = 40\%$

Test for overall effect: $Z = 0.14$ ($P = 0.89$)



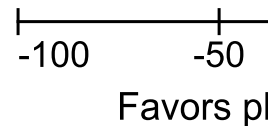
| Study or Subgroup | Weight | Mean Difference IV, Random, 95% CI |
|---|---------------|---|
| Chanet 2017 (69) | 11.0% | 2.20 [0.48, 3.92] |
| Leenders 2013 (16) | 6.7% | -1.92 [-4.47, 0.63] |
| Ottestad 2017 (74) | 16.9% | 0.60 [-0.42, 1.62] |
| Seino 2018 (75) | 16.8% | -0.40 [-1.43, 0.63] |
| ten Haaf 2019 (23) | 13.8% | -1.00 [-2.36, 0.36] |
| Tieland 2012 (28) | 8.8% | 0.00 [-2.08, 2.08] |
| Tieland 2012a (29) | 10.2% | 1.80 [-0.04, 3.64] |
| Zhu 2015 (27) | 15.7% | 0.44 [-0.71, 1.59] |
| Total (95% CI) | 100.0% | 0.26 [-0.51, 1.04] |
| Heterogeneity: $\text{Tau}^2 = 0.65$; $\text{Chi}^2 = 15.81$, $\text{df} = 7$ ($P = 0.03$); I^2 | | |
| Test for overall effect: $Z = 0.67$ ($P = 0.51$) | | |

| | | |
|-------------------------------|-------|-----------------------|
| Smithson 2018 (51) | 8.0% | 1.00 [-21.01, 22.01] |
| Pihoker 2019 - after EX (55) | 5.9% | -7.60 [-28.37, 13.17] |
| Pihoker 2019 - before EX (55) | 5.2% | 4.70 [-18.03, 27.43] |
| Rozenek 2002 (58) | 6.4% | 21.10 [1.47, 40.73] |
| Snijders 2015 (60) | 8.1% | 7.00 [-9.11, 23.11] |
| Taylor 2015 (61) | 6.4% | 5.00 [-14.49, 24.49] |
| Vangsoe 2018 (62) | 12.0% | -5.00 [-15.45, 5.45] |

Total (95% CI) **100.0%** **5.80 [-0.33, 11.93]**

Heterogeneity: $\tau^2 = 52.89$; $\chi^2 = 24.77$, $df = 15$ ($P = 0.05$); $I^2 = 39\%$

Test for overall effect: $Z = 1.86$ ($P = 0.06$)



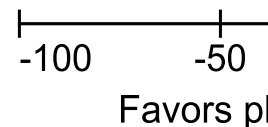
B

| Study or Subgroup | Weight | Mean Difference | |
|----------------------|--------|------------------------|----------------|
| | | IV, Random, 95% CI | I ² |
| Bell 2017 (66) | 22.3% | -1.00 [-11.06, 9.06] | |
| Bemben 2010 (67) | 5.6% | 21.70 [1.69, 41.71] | |
| Chale 2013 (68) | 11.3% | 5.20 [-8.93, 19.33] | |
| Leenders 2013 (16) | 8.7% | 0.85 [-15.29, 16.99] | |
| Ottestad 2017 (74) | 20.9% | -0.50 [-10.90, 9.90] | |
| Tieland 2012 (28) | 10.0% | 3.00 [-12.04, 18.04] | |
| Tieland 2012a (29) | 13.6% | -1.00 [-13.91, 11.91] | |
| Verdijk 2009 (76) | 7.3% | 2.00 [-15.55, 19.55] | |
| Villanueva 2014 (77) | 0.3% | 30.50 [-51.54, 112.54] | |

Total (95% CI) **100.0%** **1.97 [-2.78, 6.72]**

Heterogeneity: $\tau^2 = 0.00$; $\chi^2 = 5.19$, $df = 8$ ($P = 0.74$); $I^2 = 0\%$

Test for overall effect: $Z = 0.81$ ($P = 0.42$)



| | | |
|--------------------------|--------------|--------------------------|
| Villanueva 2014 (77) | 0.7% | 0.85 [-1.47, 3.17] |
| Volek 2013 - soy (63) | 3.8% | -0.50 [-1.48, 0.48] |
| Volek 2013 - whey (63) | 3.8% | 1.00 [0.02, 1.98] |
| Subtotal (95% CI) | 25.7% | 0.51 [0.13, 0.89] |

Heterogeneity: $\tau^2 = 0.00$; $\chi^2 = 8.12$, $df = 8$ ($P = 0.42$); $I^2 = 1\%$

Test for overall effect: $Z = 2.66$ ($P = 0.008$)

Before exercise and after exercise

| | | |
|---|--------------|--------------------------|
| Hoffman 2009 - before and after EX (19) | 0.3% | 0.80 [-2.46, 4.06] |
| Josse 2010 (50) | 11.8% | 0.80 [0.25, 1.35] |
| Taylor 2015 (61) | 3.1% | 1.10 [0.03, 2.17] |
| Verdijk 2009 (76) | 2.4% | 0.10 [-1.12, 1.32] |
| Walker 2010 (64) | 1.7% | 0.30 [-1.16, 1.76] |
| Weisgarber 2012 (65) | 0.6% | 0.20 [-2.29, 2.69] |
| Subtotal (95% CI) | 20.0% | 0.70 [0.28, 1.13] |

Heterogeneity: $\tau^2 = 0.00$; $\chi^2 = 2.04$, $df = 5$ ($P = 0.84$); $I^2 = 0\%$

Test for overall effect: $Z = 3.24$ ($P = 0.001$)

Not around exercise

| | | |
|---|--------------|--------------------------|
| Bell 2017 (66) | 6.1% | 1.37 [0.60, 2.14] |
| Chanet 2017 (69) | 2.9% | 0.50 [-0.62, 1.62] |
| Daly 2014 (70) | 6.0% | 0.50 [-0.27, 1.27] |
| Hoffman 2009 - morning and evening (19) | 0.4% | 1.70 [-1.44, 4.84] |
| Leenders 2013 - female (16) | 3.0% | 0.20 [-0.90, 1.30] |
| Leenders 2013 - male (16) | 2.4% | 0.40 [-0.84, 1.64] |
| Norton 2016 (73) | 15.2% | 0.61 [0.12, 1.10] |
| Ottestad 2017 (74) | 12.2% | 0.00 [-0.54, 0.54] |
| Snijders 2015 (60) | 2.4% | 0.20 [-1.01, 1.41] |
| Tieland 2012 (28) | 2.0% | 0.10 [-1.23, 1.43] |
| Tieland 2012a (29) | 1.7% | 1.60 [0.15, 3.05] |
| Subtotal (95% CI) | 54.3% | 0.52 [0.22, 0.82] |

Heterogeneity: $\tau^2 = 0.04$; $\chi^2 = 12.02$, $df = 10$ ($P = 0.28$); $I^2 = 17\%$

Test for overall effect: $Z = 3.42$ ($P = 0.0006$)

Total (95% CI) **100.0%** **0.55 [0.36, 0.74]**

Heterogeneity: $\tau^2 = 0.00$; $\chi^2 = 22.81$, $df = 25$ ($P = 0.59$); $I^2 = 0\%$

Test for overall effect: $Z = 5.66$ ($P < 0.00001$)

Test for subgroup differences: $\chi^2 = 0.54$, $df = 2$ ($P = 0.76$), $I^2 = 0\%$



| Study or Subgroup | Weight | Mean Difference IV, Random, |
|---|---------------|--|
| Adechian 2012 - casein (34) | 22.3% | 0.80 [-0.99] |
| Adechian 2012 - milk protein (34) | 17.1% | -0.44 [-2.52] |
| Hudson 2017 (11) | 47.1% | -1.00 [-2.11] |
| Kim, 2018 (2) | 13.4% | 0.60 [-1.78] |
| Total (95% CI) | 100.0% | -0.29 [-1.20] |
| Heterogeneity: $\text{Tau}^2 = 0.14$; $\text{Chi}^2 = 3.53$, $\text{df} = 3$ ($P = 0.32$); $I^2 = 69\%$ | | |
| Test for overall effect: $Z = 0.62$ ($P = 0.54$) | | |

| Study or Subgroup | Weight | Mean Difference IV, Random, 95% CI |
|--|---------------|---|
| At breakfast | | |
| Chanet 2017 (69) | 13.4% | 2.20 [0.48, 3.92] |
| Leenders 2013 (16) | 8.4% | -1.92 [-4.47, 0.63] |
| Zhu 2015 (27) | 18.5% | 0.44 [-0.71, 1.59] |
| Subtotal (95% CI) | 40.3% | 0.44 [-1.46, 2.33] |
| Heterogeneity: $\text{Tau}^2 = 1.97$; $\text{Chi}^2 = 7.15$, $\text{df} = 2$ ($P = 0.03$); $I^2 = 75\%$ | | |
| Test for overall effect: $Z = 0.45$ ($P = 0.65$) | | |
| At breakfast plus other timing | | |
| Ottestad 2017 (74) | 19.8% | 0.60 [-0.42, 1.62] |
| ten Haaf 2019 (23) | 16.4% | -1.00 [-2.36, 0.36] |
| Tieland 2012 (28) | 10.9% | 0.00 [-2.08, 2.08] |
| Tieland 2012a (29) | 12.6% | 1.80 [-0.04, 3.64] |
| Subtotal (95% CI) | 59.7% | 0.30 [-0.81, 1.40] |
| Heterogeneity: $\text{Tau}^2 = 0.66$; $\text{Chi}^2 = 6.47$, $\text{df} = 3$ ($P = 0.09$); $I^2 = 68\%$ | | |
| Test for overall effect: $Z = 0.53$ ($P = 0.60$) | | |
| Total (95% CI) | 100.0% | 0.39 [-0.50, 1.29] |
| Heterogeneity: $\text{Tau}^2 = 0.78$; $\text{Chi}^2 = 13.96$, $\text{df} = 6$ ($P = 0.03$); $I^2 = 69\%$ | | |
| Test for overall effect: $Z = 0.86$ ($P = 0.39$) | | |
| Test for subgroup differences: $\text{Chi}^2 = 0.02$, $\text{df} = 1$ ($P = 0.90$), $I^2 = 0\%$ | | |

| | | |
|------------------------------|------|------------------------|
| Negro 2014 (33) | 4.8% | -1.50 [-21.40, 18.40] |
| Pihoker 2019 - after EX (55) | 4.3% | -7.60 [-28.37, 13.17] |
| Villanueva 2014 (77) | 0.3% | 30.50 [-51.54, 112.54] |

Subtotal (95% CI) **14.9%** **10.92 [-7.09, 28.92]**

Heterogeneity: $\tau^2 = 199.40$; $\chi^2 = 8.43$, $df = 4$ ($P = 0.08$); $I^2 = 53\%$

Test for overall effect: $Z = 1.19$ ($P = 0.23$)

Before exercise and after exercise

| | | |
|------------------------------------|------|-----------------------|
| Herda 2013 - bioenhanced whey (45) | 3.7% | -9.00 [-31.66, 13.66] |
| Herda 2013 - whey (45) | 3.9% | -3.80 [-25.80, 18.20] |
| Hulmi 2009 (48) | 6.4% | 1.50 [-14.64, 17.64] |
| Josse 2010 (50) | 4.4% | 29.04 [8.66, 49.41] |
| Taylor 2015 (61) | 4.7% | 5.00 [-14.49, 24.49] |
| Verdijk 2009 (76) | 5.6% | 2.00 [-15.55, 19.55] |

Subtotal (95% CI) **28.6%** **4.33 [-5.52, 14.18]**

Heterogeneity: $\tau^2 = 52.78$; $\chi^2 = 7.69$, $df = 5$ ($P = 0.17$); $I^2 = 35\%$

Test for overall effect: $Z = 0.86$ ($P = 0.39$)

Not around exercise

| | | |
|--------------------|-------|-----------------------|
| Bell 2017 (66) | 11.8% | -1.00 [-11.06, 9.06] |
| Leenders 2013 (16) | 6.4% | 0.85 [-15.29, 16.99] |
| Ottestad 2017 (74) | 11.4% | -0.50 [-10.90, 9.90] |
| Rozenek 2002 (58) | 4.7% | 21.10 [1.47, 40.73] |
| Snijders 2015 (60) | 6.4% | 7.00 [-9.11, 23.11] |
| Tieland 2012 (28) | 7.1% | 3.00 [-12.04, 18.04] |
| Tieland 2012a (29) | 8.8% | -1.00 [-13.91, 11.91] |

Subtotal (95% CI) **56.5%** **1.95 [-3.06, 6.96]**

Heterogeneity: $\tau^2 = 0.00$; $\chi^2 = 4.81$, $df = 6$ ($P = 0.57$); $I^2 = 0\%$

Test for overall effect: $Z = 0.76$ ($P = 0.45$)

Total (95% CI) **100.0%** **3.93 [-0.75, 8.62]**

Heterogeneity: $\tau^2 = 21.97$; $\chi^2 = 21.96$, $df = 17$ ($P = 0.19$); $I^2 = 23\%$

Test for overall effect: $Z = 1.64$ ($P = 0.10$)

Test for subgroup differences: $\chi^2 = 0.98$, $df = 2$ ($P = 0.61$), $I^2 = 0\%$



The role of protein intake and its timing on body composition and muscle function in healthy adults: a systematic review and meta-analysis of randomized controlled trials

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SUPPLEMENTARY TABLE 1 Search strategy as used in the systematic review on 13 March 2019 for studies investigating the effect of protein intake on healthy adults

| Database | PICO | Search code |
|----------|------------------------------------|--|
| Pubmed | Population | ((("Human"[All Fields] OR "Humans"[All Fields] OR "Humans"[Mesh]) AND ("Adult"[All Fields] OR "Adults"[All Fields] OR "Adults"[Mesh]) AND ("Rat"[Title] OR "rats"[Title] OR "mouse"[Title] OR "mice"[Title] OR "rodent"[Title] OR "rodents"[Title] OR "child"[Title] OR "children"[Title] OR "childhood"[Title] OR "adolescent"[Title] OR "adolescents"[Title] OR "in vitro"[Title])) |
| | Intervention and Comparison | ((("protein"[All Fields] OR "proteins"[All Fields] OR "proteins"[Mesh:noexp] OR "essential amino acids"[All Fields] OR "amino acids, essential"[Mesh] OR "dietary protein"[All Fields] OR "dietary proteins"[Majr] OR "protein intake"[All Fields] OR "intakes"[All Fields] OR "food intake"[All Fields] OR "dietary intake"[All Fields] OR "diet"[All Fields] OR "diet"[Mesh:noexp] OR "nutrition"[All Fields] OR "nutritional"[All Fields] OR "nutritive"[All Fields] OR "nutrition"[All Fields] OR "drinking"[Mesh:noexp] OR "drink"[All Fields] OR "beverage"[All Fields] OR "beverages"[All Fields] OR "nutrient uptake"[All Fields] OR "alimentary"[All Fields] OR "alimentary"[All Fields] OR "uptake"[All Fields] OR "eat"[All Fields] OR "eating"[All Fields] OR "eating"[Mesh:noexp] OR "meals"[All Fields] OR "meal"[All Fields] OR "consumption"[All Fields] OR "consuming"[All Fields] OR "feeding"[All Fields] OR "supplement"[All Fields] OR "supplements"[All Fields] OR "supplemented"[All Fields] OR "supplementation"[All Fields] OR "supplementations"[All Fields] OR "supplements"[Majr:noexp])) AND ("timing"[All Fields] OR "time"[All Fields] OR "time"[Mesh:noexp] OR "time factors"[Mesh:noexp] OR "presleep"[All Fields] OR "pre sleep"[All Fields] OR "evening"[All Fields] OR "nighttime"[All Fields] OR "night time"[All Fields] OR "distribution"[All Fields] OR "distributions"[All Fields] OR "breakfast"[All Fields] OR "Lunch"[All Fields] OR "snack"[All Fields] OR "dinner"[All Fields] OR "dining"[All Fields])) |
| | Outcome | ((("muscle"[All Fields] OR "muscles"[All Fields] OR "muscle, skeletal"[Mesh:noexp] OR "myofibril"[All Fields] OR "myofibrils"[Mesh:noexp] OR "muscle fibril"[All Fields] OR "muscle fibrils"[All Fields]) AND ("function"[All Fields] OR "strength"[All Fields] OR "mass"[All Fields] OR "quality"[All Fields] OR "synthesis"[All Fields] OR "contractility"[All Fields] OR ("muscle strength"[Mesh] OR "Muscle Contraction"[Mesh:noexp] OR "hand strength"[All Fields] OR "grip strength"[All Fields] OR "leg press"[All Fields] OR "leg curl"[All Fields] OR "body composition"[Mesh:noexp] OR "photon absorptiometry"[All Fields] OR "absorptiometry, photon"[Mesh] OR "electric impedance"[Mesh:noexp] OR "impedance"[All Fields] OR "bioelectrical impedance"[All Fields] OR "lean body mass"[All Fields] OR "fat free mass"[All Fields] OR "lean tissue mass"[All Fields])) |
| | Clinical trial filter ¹ | (randomized controlled trial [pt] OR controlled clinical trial [pt] OR randomized [tiab] OR placebo [tiab] OR trial [tiab] OR groups [tiab]) NOT (animals [mh] NOT humans [mh]) |
| Embase | Population | ('human' OR 'humans' OR 'human'/exp) AND ('adult' OR 'adults' OR 'adult'/de) NOT ('rat':ti OR 'rats':ti OR 'rodents':ti OR 'animal':ti OR 'animals':ti OR 'child':ti OR 'children':ti OR 'childhood':ti OR 'adolescent':ti OR 'infants':ti OR 'in vitro':ti) |
| | Intervention and Comparison | ('protein' OR 'proteins' OR 'protein'/de OR 'essential amino acid' OR 'essential amino acids' OR 'essential amino acids'/de OR 'protein intake' OR 'protein intake'/exp) AND ('intake' OR 'intakes' OR 'food intake' OR 'diet' OR 'diets' OR 'dietary' OR 'diet'/de OR 'nutrition' OR 'nutritional' OR 'nutritive' OR 'nutrition'/de OR 'drinking' OR 'drinking'/de OR 'drink' OR 'beverage' OR 'beverages' OR 'beverage'/de OR 'nutrient uptake' OR 'alimentary' OR 'alimentary'/de OR 'uptake' OR 'uptakes' OR 'eat' OR 'eating' OR 'eating'/de OR 'meal' OR 'consumption' OR 'consuming' OR 'consumption'/exp OR 'feeding' OR 'feeding'/de OR 'supplement' OR 'supplements' OR 'supplementing' OR 'supplemented' OR 'supplementation' OR 'supplementation'/de OR 'supplement'/mj) AND ('timing' OR 'timing'/de OR 'time' OR 'time'/de OR 'time factor' OR 'time factors' OR 'presleep' OR 'evening' OR 'evenings' OR 'evening'/de OR 'nighttime' OR 'night time' OR 'nighttime'/de OR 'breakfast' OR 'lunch' OR 'snack' OR 'dinner' OR 'dining' OR 'supper') |

SUPPLEMENTARY TABLE 1 Search strategy as used in the systematic review on 13 March 2019 for studies investigating the effect of protein on muscle mass in healthy adults (cont.)

| Database | PICO | Search code |
|----------------|------------------------------------|---|
| Embase | Outcome | ('muscle' OR 'muscles' OR 'skeletal muscle'/de OR 'myofibril' OR 'myofibrils' OR 'muscle fibril' OR 'function' OR 'functionality' OR 'strength' OR 'mass' OR 'quality' OR 'synthesis' OR 'contraction' OR 'muscle contraction'/de OR 'muscle function'/de OR 'muscle quality'/de OR 'hand strength' OR 'grip'/exp OR 'grip strength' OR 'grip strength'/exp OR 'leg press' OR 'leg press'/exp OR 'leg cu' OR 'photon absorptiometry' OR 'photon absorptiometry'/de OR 'electric impedance' OR 'impe' OR 'impedance' OR 'bia' OR 'lean body weight' OR 'lean body weight'/de OR 'lean body mass' OR 'tissue mass' OR 'lean tissue mass'/de |
| | Clinical trial filter ¹ | 'crossover procedure':de OR 'double-blind procedure':de OR 'randomized controlled trial':de OR 'factorial*' OR crossover* OR cross NEXT/1 over* OR placebo* OR doubl* NEAR/1 blind* OR volunteer*):de,ab,ti |
| CINAHL | Population | S1: "human" OR "humans" OR (MH "Human") , S2: "adult" OR "adults" OR (MH "Adult+") , S3: TI rodent OR TI rodents OR TI animal OR TI animals OR TI child OR TI children OR TI childh OR infant OR TI infants OR TI "in vitro" S4: S1 AND S2 NOT S3 |
| | Intervention and Comparison | S5: "protein" OR "proteins" OR (MH "Proteins") OR "essential amino acid" OR "essential amin" OR "dietary protein" OR (MM "Dietary Proteins+") OR "protein intake" , S6: "intake" OR "intakes" OR "dietary intake" OR "diet" OR "diets" OR "dietary" OR (MH "Diet") OR "nutrition" OR "nutritional pattern" OR "drinking" OR "drink" OR "beverage" OR "beverages" OR (MH "Beverages") OR "uptake" OR "uptakes" OR "eat" OR "eating" OR (MH "Eating") OR "meals" OR "meal" OR "consuming" OR "feeding" OR "supplement" OR "supplements" OR "supplementing" OR "supplementations" OR "supplementary" OR (MM "Dietary Supplements") , S7: "timing" OR "factors" OR (MH "Time Factors") OR "presleep" OR "pre sleep" OR "evening" OR "evenings" OR "distributions" OR "time-of-day" OR "breakfast" OR "Lunch" OR "snack" OR "dinner" OR "supper" S8: S5 AND S6 AND S7 |
| | Outcome | S9: "muscle" OR "muscles" OR (MH "Muscle, Skeletal") OR "myofibril" OR "myofibrils" OR "muscle functionality" OR "strength" OR "mass" OR "quality" OR "synthesis" OR "contraction" OR "arm strength" OR "Muscle Contraction") OR "hand strength" OR "hand grip" OR "grip strength" OR (MH "Grip Strength") OR "composition" OR (MH "Body Composition") OR "photon absorptiometry" OR (MH "Absorption") OR "Electric Impedance") OR "impedance" OR "bioelectrical impedance" OR "bia" OR "lean body mass" OR (MH "Fat Free Mass") OR "lean tissue mass" S12: (S9 AND S10) OR S11 |
| | Clinical trial filter ¹ | TX allocat* random* OR (MH "Quantitative Studies") OR (MH "Placebos") OR TX placebo* OR TX Assignment") OR TX randomi* control* trial* OR TX ((singl* n1 blind*) OR (singl* n1 mask*)) OR TX ((tripl* n1 blind*) OR (tripl* n1 mask*)) OR TX ((trebl* n1 blind*) OR (trebl* n1 mask*)) (MH "Clinical Trials+") |
| Web of Science | Population | ALL FIELDS: ("human" OR "humans") AND ALL FIELDS: ("adult" OR "adults") NOT TITLE: "rodent" OR "rodents" OR "animal" OR "animals" OR "child" OR "children" OR "childhood" OR "infants" OR "in vitro") |

SUPPLEMENTARY TABLE 1 Search strategy as used in the systematic review on 13 March 2019 for studies investigating the effect of protein intake on healthy adults (cont.)

| Database | PICO | Search code |
|----------------|------------------------------------|--|
| Web of Science | Intervention and Comparison | ALL FIELDS: ("protein" OR "proteins" OR "essential amino acid" OR "essential amino acids" FIELDS: ("intake" OR "intakes" OR "food intake" OR "dietary intake" OR "diet" OR "diets" OR "nutritive" OR "dietary pattern" OR "drinking" OR "drink" OR "beverage" OR "beverages" OR "OR "uptake" OR "uptakes" OR "eat" OR "eating" OR "meals" OR "meal" OR "consumption" C "supplements" OR "supplementing" OR "supplemented" OR "supplementation" OR "supplem FIELDS: ("timing" OR "time" OR "time factor" OR "time factors" OR "presleep" OR "pre sleep" "night time" OR "distribution" OR "distributions" OR "time-of-day" OR "breakfast" OR "lunch" C |
| | Outcome | ALL FIELDS: ("muscle" OR "muscles" OR "myofibril" OR "myofibrils" OR "muscle fibril" OR "r "functionality" OR "strength" OR "mass" OR "quality" OR "synthesis" OR "contraction" OR "an "grip strength" OR "leg press" OR "leg curl" OR "body composition" OR "photon absorptiomet "bioelectric impedance" OR "bia" OR "lean body weight" OR "lean body mass" OR "fat free m |
| | Clinical trial filter ¹ | TS=clinical trial* OR TS=research design OR TS=comparative stud* OR TS=evaluation stud* OR TS=prospective stud* OR TS=random* OR TS=placebo* OR TS=(single blind*) OR TS=(|

¹ The following filters were used: PubMed filter from Cochrane - Max Sensitivity, EMBASE.com filter from Cochrane, CINAHL filter from SIGN. All filters can be found at <http://aub.edu.lb/libguides.com/c.php?g=329862&p=3023731>

SUPPLEMENTARY TABLE 2 Characteristics of studies investigating the effect of protein intake on body composition and muscle strength in

| Study | Group | n (M, F) | Age, y ² | BMI, kg · m ⁻² ² | Physical activity status | Duration, wk | Energy restriction | Exercise intervention | Protein type | Servings, n/d | Fat gain, g |
|--------------------|-------|-------------|---------------------|--|--------------------------|--------------|--------------------|------------------------|---|---------------|-------------|
| Adechian 2012 (1) | P1 | 10 (2, 8) | 35.1 ± 1.5 | 32.0 ± 0.8 | NR | 6 | BMR level | - | C | 4 | 8 |
| | P1 | 10 (2, 8) | 34.6 ± 1.4 | 33.5 ± 1.4 | NR | 6 | BMR level | - | C | 4 | 8 |
| | P2 | 10 (1, 9) | 30.6 ± 2.3 | 32.9 ± 1.4 | NR | 6 | BMR level | - | MP | 4 | 8 |
| Baer 2011 (2) | P2 | 11 (3, 8) | 33.6 ± 1.8 | 31.9 ± 0.9 | NR | 6 | BMR level | - | MP | 4 | 8 |
| | P1 | 23 (10, 13) | 49 ± 9 | 31.0 ± 2.2 | NR | 23 | - | - | W | 2 | 5 |
| | P2 | 25 (12, 13) | 53 ± 9 | 30.9 ± 2.3 | NR | 23 | - | - | S | 2 | 5 |
| Hudson 2017 (3) | CON | 25 (12, 13) | 51 ± 9 | 31.1 ± 2.5 | NR | 23 | - | - | - | 2 | - |
| | P | 20 (9, 11) | 36 ± 2 | 30.8 ± 0.5 | NR | 16 | 750 kcal | RT 3x per wk | Animal food sources | 3 | 9 |
| Johnston 2017 (4) | P | 21 (6, 15) | 33 ± 2 | 31.9 ± 0.5 | NR | 16 | 750 kcal | RT 3x per wk | Animal food sources | 3 | 9 |
| | HP | 10 (5, 5) | 41.9 (12.6) | 33.7 (4.7) | NR | 6 | 500 kcal | - | Pasta and flaked cereal enriched with W, wheat gluten, and EW | 3 | N |
| | LP | 11 (1, 10) | 45.6 (12.0) | 30.8 (7.6) | NR | 6 | 500 kcal | - | - | 3 | N |
| Mojtahedi 2011 (5) | P | 13 (0, 13) | 64.7 (4.4) | 32.3 (3.9) | UT | 24 | 500 kcal | AT + ST 2-3x per wk | W | 2 | 5 |
| | CON | 13 (0, 13) | 64.6 (5.2) | 32.7 (4.2) | UT | 24 | 500 kcal | AT + ST 2-3x per wk | - | 2 | - |
| Murphy 2018 (6) | P1 | 10 (10, 0) | 66 (4) | 31 (5) | NR | 4 | 300 kcal | RT 3x per wk from wk 3 | AV food sources + W | 4 | 5 |
| | P2 | 10 (10, 0) | 66 (4) | 31 (5) | NR | 4 | 300 kcal | RT 3x per wk from wk 3 | AV food sources | 4 | - |
| Ormsbee 2015 (7) | P1 | 13 (0, 13) | 29.3 ± 1.2 | 34.4 ± 1.3 | UT | 4 | - | RT + HIIT 3x per wk | W | 1 | 3 |
| | P2 | 14 (0, 14) | 30.0 ± 1.9 | 36.5 ± 1.8 | UT | 4 | - | RT + HIIT 3x per wk | C | 1 | 3 |
| | CON | 10 (0, 10) | 27.7 ± 2.3 | 33.1 ± 1.7 | UT | 4 | - | RT + HIIT 3x per wk | - | 1 | - |
| Pal 2010 (8) | P1 | 25 (3, 22) | 18-65 ⁴ | 32.0 ± 0.8 | | 12 | - | - | W | 2 | 5 |
| | P2 | 20 (3, 17) | 18-65 ⁴ | 31.3 ± 0.9 | | 12 | - | - | C | 2 | 5 |
| | CON | 25 (4, 21) | 18-65 ⁴ | 30.6 ± 0.9 | | 12 | - | - | - | 2 | - |
| Smith 2018 (9) | HP | 25 (0, 25) | 50-65 ⁴ | 30-50 ⁴ | UT | 24 | 30% TEE | - | W | 2 | N |
| | LP | 27 (0, 27) | 50-65 ⁴ | 30-50 ⁴ | UT | 24 | 30% TEE | - | - | - | - |
| | NP | 18 (0, 18) | 50-65 ⁴ | 30-50 ⁴ | UT | 24 | - | - | - | - | - |

SUPPLEMENTARY TABLE 2 Characteristics of studies investigating the effect of protein intake on body composition and muscle strength in

| Study | Group | n (M, F) | Age, y ² | BMI, kg · m ⁻² ² | Physical activity status | Duration, wk | Energy restriction | Exercise intervention | Protein type | Servings, n/d | Protein source |
|---------------------|-------|-------------|---------------------|--|--------------------------|--------------|--------------------|-----------------------|---|---------------|----------------|
| Verreijen 2015 (10) | P | 30 (14, 16) | 63.7 (6.0) | 32.7 (3.1) | NR | 13 | 600 kcal | RT 3x per wk | W + Leu | 1 - 2 | 2 |
| | CON | 30 (14, 16) | 63.0 (6.0) | 33.3 (4.3) | NR | 13 | 600 kcal | RT 3x per wk | - | 2 | - |
| Wright 2018 (11) | HP | 12 (7, 5) | 70 (6) | 32.2 (3.4) | UT | 12 | - | - | Eggs | 3 | 5 |
| | LP | 10 (5, 5) | 71 (3) | 30.5 (3.3) | UT | 12 | - | - | - | 3 | - |
| Wycherley 2012 (12) | HP | 33 (33, 0) | 51.3 (9.4) | 33.8 ⁵ | - | 52 | Yes - NR | - | Low-fat dairy products, lean meat, and fish | 3 | 1 |

¹ A, afternoon; AV, animal and vegetable; AE, after exercise; AT, aerobic training; B, breakfast; BB, before breakfast; BD, before dinner; BM, before meal; BS, before sleep; C, casein; CHO, carbohydrate; CON, control group; D, dinner; E, evening; EV, evenly; EX, exercise; F, female; HIIT, high-intensity interval training; I, insulin; L, lunch; Leu, Leucine; LP, low protein diet; M, male; MP, milk protein; NP, no protein supplemented; NR, not reported; P, protein; RT, resistance training; T, total; TE, total energy expenditure; UT, untrained; W, whey protein.

² Data presented as mean (SD), mean ± SEM, or range.

³ Total protein intake per day presented as diet or as diet plus supplementation if the latter was provided.

⁴ No group information available and overall values used.

⁵ Data calculated as the mean weight (kg) divided by the square of mean height (m²).

SUPPLEMENTARY TABLE 3 Summary of conclusions from studies investigating the effect of protein intake on lean body mass in adults, older adults, and obese

| | Author, year | Sex | Protein intervention (type, amount, duration) | Other interventions |
|---------------------|----------------------|------|---|-------------------------|
| Adults | Arciero 2016 (13) | F | W bars, 2.0 g · kg ⁻¹ · d ⁻¹ , 12 wk | RISE 4x per wk |
| | Burke 2001 (14) | M | W+Cr or only W, 1.2 to 1.3 g · kg ⁻¹ · d ⁻¹ , 6 wk | RT 4x per wk |
| | Hoffman 2009 (15) | M | CG+W+C, 42 g · d ⁻¹ , 10wk | RT 4x per wk |
| | Hwang 2017 (16) | M | W, 25 g · d ⁻¹ , 10 wk | RT 4x per wk |
| | Josse 2010 (17) | F | Fat-free milk, 36 g · d ⁻¹ , 12 wk | RT 5x per wk |
| | Negro 2014 (18) | M, F | Lean beef, 20 g · d ⁻¹ , 8 wk | RT 3x per wk |
| | Ormsbee 2018 (19) | M, F | Mixed protein shake, 84 g · d ⁻¹ , 24 wk | AT + RT 5x per wk |
| | Pihoker 2019 (20) | F | Mixed protein shake, 25 g · d ⁻¹ , 6 wk | RT 2x per wk |
| | Reidy 2016 (21) | M | W+S+C or W only, 22 g · d ⁻¹ , 12 wk | RT 3x per wk |
| | Schoenfeld 2017 (22) | M | Wh, 25 g · d ⁻¹ , 10 wk | RT 3x per wk |
| | Snijders 2015 (23) | M | Ch+C, 27.5 g · d ⁻¹ , 12 wk | RT 3x per wk |
| | Taylor 2016 (24) | F | W, 48 g · d ⁻¹ , 8 wk | AgT + ET + RT 4x per wk |
| | Volek 2013 (25) | M, F | W or S, ~20 g · d ⁻¹ , 36 wk | RT 3x per wk |
| | Walker 2010 (26) | M | W+Leu, 51.8 g · d ⁻¹ , 8 wk | AT + RT ≥3x per wk |
| | Weisgarber 2012 (27) | M, F | W, 0.3 g · kg ⁻¹ · d ⁻¹ , 8 wk | RT 4x per wk |
| Older adults | Bell 2017 (28) | M | W+Cr, 60 g · d ⁻¹ , 20 wk | RT 2x per wk from wk 7 |
| | Chale 2013 (29) | M, F | W, 40 g · d ⁻¹ , 24 wk | RT 3x per wk |
| | Chanet 2017 (30) | M | W+Leu, 20 g · d ⁻¹ , 6 wk | Only protein intake |
| | Daly 2014 (31) | F | Lean red meat, 45g · d ⁻¹ , 16 wk | AgT + RT 2x per wk |
| | de Branco 2019 (32) | F | W, 30 g · d ⁻¹ , 8 wk | RT 3x per wk |
| | Esmarck 2001 (33) | M | MP+S, 10 g · d ⁻¹ , 12 wk | RT 3x per wk |
| | Holm 2008 (34) | F | W, 10 g · d ⁻¹ , 24 wk | RT 3x per wk |
| | Kim 2018 (35) | M, F | Egg, dairy, and beef, diet with 1.1 g · kg ⁻¹ · d ⁻¹ , 8 wk | Only protein intake |
| | Leenders 2013 (36) | M, F | W+C, 15 g · d ⁻¹ , 24 wk | RT 3x per wk |
| | Norton 2016 (37) | M, F | MP, 0.3 g · kg ⁻¹ · d ⁻¹ , 24 wk | Only protein intake |
| | Ottestad 2017 (38) | M, F | Protein-enriched milk, 40.8 g · d ⁻¹ , 12 wk | Only protein intake |
| | ten Haaf 2019 (39) | M, F | MP, 31 g · d ⁻¹ , 12 wk | AT ?x per wk |
| | Tieland 2012 (40) | M, F | MP, 30 g · d ⁻¹ , 24 wk | Only protein intake |
| | Tieland 2012a (41) | M, F | MP, 30 g · d ⁻¹ , 24 wk | RT 2x per wk |
| | Verdijk 2009 (42) | M | Ch, 20 g · d ⁻¹ , 12 wk | RT 3x per wk |
| | Villanueva 2014 (43) | M | W+Cr, 35 g · d ⁻¹ , 12 wk | RT 3x per wk |
| Obese | Adechian 2012 (1) | M, F | C or MP, total diet with 87 g · d ⁻¹ , 6 wk | ER at BMR level |

SUPPLEMENTARY TABLE 3 Summary of conclusions from studies investigating the effect of protein intake on lean body mass in adults, older adults, and children

| Author, year | Sex | Protein intervention (type, amount, duration) | Other interventions |
|------------------|------|---|--|
| Hudson 2017 (3) | M, F | Animal food sources, total diet with 90 g · d ⁻¹ , 16 wk | RT 3x per wk + ER 750 kcal · d ⁻¹ |
| Ormsbee 2015 (7) | F | W or C, 30 g · d ⁻¹ , 4 wk | RT + HIIT 3x per wk |
| Pal 2010 (8) | M, F | W or C, 54 g · d ⁻¹ , 12 wk | Only protein intake |
| Smith 2018 (9) | F | W, total diet with 1.2 g · kg ⁻¹ · d ⁻¹ , 24 wk | ER of 30% TEE |
| Wright 2018 (11) | M, F | Animal and vegetable food sources, 50 g · d ⁻¹ , 12 wk | Only protein intake |

¹ Arrows indicate the direction of change in lean body mass. →, no significant difference; ↑, significant increase; ↓, significant decrease; ↗, significant but <0.5 kg; AE, after exercise; AgT, agility training; AT, aerobic training; BE, before exercise; BMR, basal metabolic rate; C, casein; E, evening; ER, energy restriction; ET, explosive training; EV, evenly; F, female; HIIT, high-intensity interval training; Leu, Leucine; M, male; interval + stretching + endurance training; RT, resistance training; S, soy; SK, skewed; TEE, total energy expenditure; W, whey protein; Wh, whole milk; ² Only graph representation available.

SUPPLEMENTARY TABLE 4 Summary of conclusions from studies investigating the effect of protein intake on body composition in adults, lean body mass ¹

| | Author, year | Sex | Protein intervention (type, amount, duration) | Other interventions | Absolute protein g |
|-------------|---------------------|------|--|--|--------------------------|
| FFM | Gryson 2014 (44) | M | C+MP, 10 vs. 4 g · d ⁻¹ , 16 wk | AT + RT 3x per wk | ↑ |
| | Hida 2012 (45) | F | EW, 15 g · d ⁻¹ , 8 wk | TS ≥6x per wk | ↑ |
| | Johnston 2017 (4) | M, F | Pasta and flaked cereal enriched with W, wheat gluten, and EW; diet with 1.4 g · kg ⁻¹ · d ⁻¹ , 6 wk | ER 500 Kcal · d ⁻¹ | ↑ |
| | Wycherley 2012 (12) | M | Low-fat dairy products, lean meat, and fish; diet with 142 g · d ⁻¹ , 52 wk | ER - Kcal not reported | ↓ |
| LST | Joy 2018 (46) | M | C, 35 g · d ⁻¹ , 10 wk | RT 4x per wk | ↑ ED; ↑ |
| | Mojtahedi 2011 (5) | F | W, 50 g · d ⁻¹ , 24 wk | AT+ST 2-3x per wk + ER 500 Kcal · d ⁻¹ | ↓ |
| | Seino 2018 (47) | M, F | Protein-enriched milk, 10.5 g · d ⁻¹ , 12 wk | RT 2x per wk | → |
| SMM | Huang 2017 (48) | M | W, 33.5 g · d ⁻¹ , 5 wk | AT 7x per wk | ↑ |
| | Lockwood 2017 (49) | M | Only W, only Wh, or W+LF; 60 g · d ⁻¹ , 8 wk | RT 4x per wk | ↑ W; ↑ W |
| | Mobley 2017 (50) | M | W+Leu, Wh+Leu, or S+Leu, 50.8 to 78.4 g · d ⁻¹ , 12 wk | RT 3x per wk | ↑ W+Leu ↑ S+Leu |
| | Nabuco 2018 (51) | F | Wh, 27 g · d ⁻¹ , 12 wk | RT 3x per wk | ↑ BE; ↑ |
| MM | Orsatti 2018 (52) | F | S+fat-free milk, 25 g · d ⁻¹ , 16 wk | RT 2-3x per wk | ↑ |
| | Vorup 2017 (53) | M, F | Low-fat milk+MP, 36 g · d ⁻¹ , 12 wk | TS 2x per wk | → |
| FBFM | Bemben 2010 (54) | M | W or W+Cr, 35 or 5 g · d ⁻¹ , 14 wk | RT 3x per wk | ↑ W; ↑ W |
| | Hartman 2007 (55) | M | Fat-free milk or fat-free soy drink, 25 g · d ⁻¹ , 12 wk | RT 5x per wk | ↑ Fat-free ↑ fat-free |
| | Vangsoe 2018 (56) | M | Insect protein bars, 0.4 g · kg ⁻¹ , 8 wk | RT 4x per wk | ↑ |

¹ Arrows indicate the direction of change in body composition parameters. →, no significant difference; ↑, significant increase; ↓, significant decrease not significant but ≥0.5 kg; AE, after exercise; AT, aerobic training; BE, before exercise; BS, before sleep; C, casein; Cr, creatine; white protein; F, female, FBFM, fat- and bone-free mass; FFM, fat-free mass, Leu, Leucine; LF, lactoferrin; LST, lean soft tissue; M, male; training; S, soy; SMM, skeletal muscle mass; ST, stretching training; TS, team sports; SK, skewed; W, whey protein; Wh, hydrolyzed whey.

SUPPLEMENTARY TABLE 5 Summary of conclusions from studies investigating the effect of protein intake on muscle cross-sectional area in adults ¹

| | Author, year | Sex | Protein intervention (type, amount, duration) | Other interventions | Variable assessed |
|--------------------|-------------------|--|--|---------------------------|---------------------------|
| CSA | Chale 2013 (29) | M, F | W, 40 g · d ⁻¹ , 24 wk | RT 3x per wk | Mid-thigh muscle |
| | Coburn 2006 (57) | M | W+Leu, 26.2 to 52.4 g · d ⁻¹ , 8 wk | RT 3x per wk | Vastus lateralis muscle |
| | | | | | Vastus medialis |
| | | | | | Vastus intermedius |
| | | | | | Rectus femoris muscle |
| | Daly 2014 (31) | F | Lean red meat, 45g · d ⁻¹ , 16 wk | AgT + RT 2x per wk | Femur muscle |
| | Esmarck 2001 (33) | M | MP+S, 10 g · d ⁻¹ , 12 wk | RT 3x per wk | Quadriceps femoris muscle |
| | Hartman 2007 (55) | M | Fat-free milk or Fat-free soy drink, 35 g · d ⁻¹ , 12 wk | RT 5x per wk | Type I muscle fiber |
| | | | | | Type II muscle fiber |
| | Herda 2013 (58) | M | bW or W, 20 to 40 g · d ⁻¹ , 8 wk | RT 3x per wk | Femur muscle |
| | Holm 2008 (34) | F | W, 10 g · d ⁻¹ , 24 wk | RT 3x per wk | Quadriceps femoris muscle |
| | | | | | Type I muscle fiber |
| | | | | | Type II muscle fiber |
| | Hulmi 2009 (59) | M | W, 30 g · d ⁻¹ , 21 wk | RT 2x per wk | Quadriceps muscle |
| | | | | | Vastus lateralis muscle |
| | | | | Vastus medialis | |
| | | | | Vastus intermedius | |
| | | | | Rectus femoris muscle | |
| Joy 2018 (46) | M | C, 35 g · d ⁻¹ , 10 wk | RT 4x per wk | Rectus femoris muscle | |
| Leenders 2013 (36) | M, F | W+C, 15 g · d ⁻¹ , 24 wk | RT 3x per wk | Quadriceps femoris muscle | |
| | | | | Type I muscle fiber | |
| | | | | Type II muscle fiber | |
| Mobley 2017 (50) | M | W+Leu, Wh+Leu, or S+Leu, 50.8 to 78.4 g · d ⁻¹ , 12 wk | RT 3x per wk | Type I muscle fiber | |
| | | | | Type II muscle fiber | |
| Reidy 2016 (21) | M | W+S+C or W only, 22 g · d ⁻¹ , 12 wk | RT 3x per wk | Knee extensor muscle | |
| Snijders 2015 (23) | M | Ch+C, 27.5 g · d ⁻¹ , 12 wk | RT 3x per wk | Quadriceps muscle | |
| | | | | Type I muscle fiber | |
| | | | | Type II muscle fiber | |

SUPPLEMENTARY TABLE 5 Summary of conclusions from studies investigating the effect of protein intake on muscle cross-sectional area and muscle thickness in healthy adults (cont.)¹

| | Author, year | Sex | Protein intervention (type, amount, duration) | Other interventions | Variable assessed |
|----|----------------------|------|--|------------------------|---|
| MT | Tieland 2012 (40) | M, F | MP, 30 g · d ⁻¹ , 24 wk | Only protein intake | Type I muscle fiber Type II muscle fiber |
| | Verdijk 2009 (42) | M | Ch, 20 g · d ⁻¹ , 12 wk | RT 3x per wk | Quadriceps muscle Type I muscle fiber Type II muscle fiber |
| | Wright 2018 (11) | M, F | Animal and vegetable food sources, 50 g · d ⁻¹ , 12 wk | Only protein intake | Thigh medial muscle |
| | Zhu 2015 (60) | F | W, 30 g · d ⁻¹ , 104 wk | Only protein intake | Calf medial muscle Calf muscle |
| | Joy 2018 (46) | M | C, 35 g · d ⁻¹ , 10 wk | RT 4x per wk | Combined MT of the vastus lateralis and vastus intermedius |
| | Mobley 2017 (50) | M | W+Leu, Wh+Leu, or S+Leu, 50.8 to 78.4 g · d ⁻¹ , 12 wk | RT 3x per wk | Vastus lateralis muscle |
| | Naclerio 2017 (61) | M | BF or W, 16.4 or 18 g · d ⁻¹ , 8 wk | RT 3x per wk | Biceps brachialis muscle Vastus medialis muscle |
| | Reidy 2016 (21) | M | W+S+C or W only, 22 g · d ⁻¹ , 12 wk | RT 3x per wk | Knee extensor muscle |
| | Schoenfeld 2017 (22) | M | Wh, 25 g · d ⁻¹ , 10 wk | RT 3x per wk | Biceps brachii muscle Triceps brachii muscle Medial quadriceps femoris muscle Lateral quadriceps femoris muscle |
| | Weisgarber 2012 (27) | M, F | W, 0.3 g · kg ⁻¹ · d ⁻¹ , 8 wk | RT 4x per wk | Elbow flexor muscle Elbow extensor muscle Knee flexor muscle Knee extensor muscle Ankle plantar flexor muscle Ankle dorsiflexor muscle |

¹ Arrows indicate the direction of change in muscle cross-sectional area and muscle thickness. →, no significant difference; ↑, significant increase; ↓, significant decrease; NS, not significant; BF, before exercise; BS, before sleep; C, casein; Cr, creatine; CSA, cross-sectional area; ED, early in the day; F, female; Leu, Leucine; L, late in the day; M, male; MT, muscle thickness; P, post-exercise; S, soy; W, whey protein.

SUPPLEMENTARY TABLE 6 Summary of conclusions from studies investigating the effect of protein intake on handgrip strength in older adults

| Author, year | Sex | Protein intervention (type, amount, duration) | Other interventions | ΔHGS |
|---------------------|------|---|--|------|
| Older adults | | | | |
| Chanet 2017 (30) | M | W+Leu, 20 g · d ⁻¹ , 6 wk | Only protein intake | ↗ |
| de Branco 2019 (32) | F | W, 30 g · d ⁻¹ , 8 wk | RT 3x per wk | ↑ |
| Kim 2018 (35) | M, F | Egg, dairy, and beef, diet with 1.1 g · kg ⁻¹ · d ⁻¹ , 8 wk | Only protein intake | ↗ |
| Leenders 2013 (36) | M, F | W+C, 15 g · d ⁻¹ , 24 wk | RT 3x per wk | → |
| Ottestad 2017 (38) | M, F | Protein-enriched milk, 40.8 g · d ⁻¹ , 12 wk | Only protein intake | → |
| Seino 2018 (47) | M, F | Protein-enriched milk, 10.5 g · d ⁻¹ , 12 wk | RT 2x per wk | → |
| ten Haaf 2019 (39) | M, F | MP, 31 g · d ⁻¹ , 12 wk | AT ?x per wk | → |
| Tieland 2012 (40) | M, F | MP, 30 g · d ⁻¹ , 24 wk | Only protein intake | → |
| Tieland 2012a (41) | M, F | MP, 30 g · d ⁻¹ , 24 wk | RT 2x per wk | → |
| Zhu 2015 (60) | F | W, 30 g · d ⁻¹ , 104 wk | Only protein intake | ↓ |
| Obese | | | | |
| Verreijen 2015 (10) | M, F | W+Leu, 23 to 46 g · d ⁻¹ , 13 wk | RT 3x per wk + ER 600 Kcal · d ⁻¹ | ↑ |

¹ Arrows indicate the direction of change in handgrip strength. →, no significant difference; ↑, significant increase; ↓, significant decrease; ↗, increase in men; ↘, decrease in men; ↖, increase in women; ↙, decrease in women. AT, aerobic training; BE, before exercise; C, casein; ER, energy restriction; EV, evenly; F, female; Leu, Leucine; M, male; MP, milk protein; R, resistance training; W, whey.

SUPPLEMENTARY TABLE 7 Summary of conclusions from studies investigating the effect of protein intake on leg press strength in adults,

| | Author, year | Sex | Protein intervention (type, amount, duration) | Other interventions | Abs pro |
|---------------------|----------------------|------|--|------------------------|------------|
| Adults | Arciero 2016 (13) | F | W bars, 2.0 g · kg ⁻¹ · d ⁻¹ , 12 wk | RISE 4x per wk | ↑ |
| | Hartman 2007 (55) | M | Fat-free milk or Fat-free soy drink, 35 g · d ⁻¹ , 12 wk | RT 5x per wk | ↑ F |
| | Herda 2013 (58) | M | bW or W, 20 to 40 g · d ⁻¹ , 8 wk | RT 3x per wk | → b |
| | Hulmi 2009 (59) | M | W, 30 g · d ⁻¹ , 21 wk | RT 2x per wk | ↑ |
| | Hwang 2017 (16) | M | W, 25 g · d ⁻¹ , 10 wk | RT 4x per wk | ↑ |
| | Josse 2010 (17) | F | Fat-free milk, 36g · d ⁻¹ , 12 wk | RT 5x per wk | ↑ |
| | Joy 2018 (46) | M | C, 35 g · d ⁻¹ , 10 wk | RT 4x per wk | ↑ E |
| | Negro 2014 (18) | M, F | Lean beef, 20 g · d ⁻¹ , 8 wk | RT 3x per wk | ↑ |
| | Ormsbee 2018 (19) | M, F | Mixed protein shake, 84 g · d ⁻¹ , 24 wk | AT + RT 5x per wk | ↑ |
| | Pihoker 2019 (20) | F | Mixed protein shake, 25 g · d ⁻¹ , 6 wk | RT 2x per wk | ↑ B |
| | Rozenek 2002 (62) | M | Protein type not report+milk, 106 g · d ⁻¹ , 8 wk | RT 4x per wk | ↑ |
| | Snijders 2015 (23) | M | Ch+C, 27.5 g · d ⁻¹ , 12 wk | RT 3x per wk | ↑ |
| | Taylor 2016 (24) | F | W, 48 g · d ⁻¹ , 8 wk | AgT + ET+ RT 4x per wk | ↑ |
| | Vangsoe 2018 (56) | M | Insect protein bars, 0.4 g · kg ⁻¹ · d ⁻¹ , 8 wk | RT 4x per wk | ↑ |
| Older adults | Bell 2017 (28) | M | W+Cr, 60 g · d ⁻¹ , 20 wk | RT 2x per wk from wk 7 | ↑ |
| | Bemben 2010 (54) | M | Only W or W+Cr, 35 or 40 g · d ⁻¹ , 14 wk | RT 3x per wk | ↑ V |
| | Chale 2013 (29) | M, F | W, 40 g · d ⁻¹ , 24 wk | RT 3x per wk | ↑ |
| | Ottestad 2017 (38) | M, F | Protein-enriched milk, 40.8 g · d ⁻¹ , 12 wk | Only protein intake | → |
| | Tieland 2012 (40) | M, F | MP, 30 g · d ⁻¹ , 24 wk | Only protein intake | ↑ |
| | Tieland 2012a (41) | M, F | MP, 30 g · d ⁻¹ , 24 wk | RT 2x per wk | ↑ |
| | Verdijk 2009 (42) | M | Ch, 20 g · d ⁻¹ , 12 wk | RT 3x per wk | ↑ |
| | Villanueva 2014 (43) | M | W+Cr, 35 g · d ⁻¹ , 12 wk | RT 3x per wk | ↑ |
| Obese | Ormsbee 2015 (7) | F | W or C, 30 g · d ⁻¹ , 4 wk | RT + HIIT 3x per wk | ↑ V |

¹ Arrows indicate the direction of change in leg press strength. →, no significant difference; ↑, significant increase; ↗, Increase not significant; BS, before sleep; bW, bio-enhanced whey; C, casein; Ch, hydrolyzed casein; Cr, creatine; ED, early in the day; ET, explosive training; F, female; MP, milk protein; RISE, resistance + interval + stretching + endurance training; RT, resistance training; W, whey protein.

² Only graph representation available.

SUPPLEMENTARY TABLE 8 Summary of conclusions from studies investigating the effect of protein intake on strength measurements in a

| Author, year | Sex | Protein intervention (type, amount, duration) | Other interventions | Bench press | Chest press and chest fly | Lat pull up |
|-------------------------------|------|---|------------------------|-------------------------------------|------------------------------------|-------------------|
| Arciero 2016 (13) | F | W bars, 2.0 g · kg ⁻¹ · d ⁻¹ , 12 wk | RISE 4x per wk | ↑→ | - | - |
| Bemben 2010 (54) ² | M | Only W or W+Cr, 35 or 40 g · d ⁻¹ , 14 wk | RT 3x per wk | ↑→ W; ↑→ W+Cr | - | ↑ |
| Burke 2001 (14) | M | Only W or W+Cr, 1.2 to 1.3 g · kg ⁻¹ · d ⁻¹ , 6 wk | RT 4x per wk | ↑→ W; ↑↑ W+Cr | - | - |
| Campbell 2018 (63) | F | W, 50 vs. 10 g · d ⁻¹ , 8 wk | RT 4x per wk | - | - | - |
| Chale 2013 (29) | M, F | W, 40 g · d ⁻¹ , 24 wk | RT 3x per wk | - | - | - |
| Coburn 2006 (57) | M | W+Leu, 26.2 to 52.4 g · d ⁻¹ , 8 wk | RT 3x per wk | - | - | - |
| Daly 2014 (31) | F | Lean red meat, 45g · d ⁻¹ , 16 wk | AgT + RT 2x per wk | - | - | - |
| de Branco 2019 (32) | F | W, 30 g · d ⁻¹ , 8 wk | RT 3x per wk | ↑ BE; ↑ AE; timing: ↗ | - | - |
| Esmarck 2001 (33) | M | MP+S, 10 g · d ⁻¹ , 12 wk | RT 3x per wk | - | - | - |
| Gryson 2014 (44) | M | C+MP, 10 vs. 4 g · d ⁻¹ , 16 wk | AT + RT 3x per wk | - | - | - |
| Hartman 2007 (55) | M | F-FM or F-FS, 25 g · d ⁻¹ , 12 wk | RT 5x per wk | - | - | - |
| Herda 2013 (58) | M | bW or W, 20 to 40 g · d ⁻¹ , 8 wk | RT 3x per wk | ↑→ bW; ↑→ W | - | - |
| Hida 2012 (45) | F | EW, 15 g · d ⁻¹ , 8 wk | TS ≥6x per wk | ↑→ | - | - |
| Hoffman 2009 (15) | M | CG+W+C, 42 g · d ⁻¹ , 10wk | RT 4x per wk | ↑→ Mo, E; ↑→ BE, AE | - | - |
| Holm 2008 (34) | F | W, 10 g · d ⁻¹ , 24 wk | RT 3x per wk | - | - | - |
| Hulmi 2009 (59) | M | W, 30 g · d ⁻¹ , 21 wk | RT 2x per wk | ↑→ | - | - |
| Josse 2010 (17) | F | Fat-free milk, 36g · d ⁻¹ , 12 wk | RT 5x per wk | ↑ | ↑↗ | ↑ |
| Joy 2018 (46) | M | C, 35 g · d ⁻¹ , 10 wk | RT 4x per wk | ↑ ED; ↑ BS; timing: → | - | - |
| Kim 2018 (35) | M, F | Egg, dairy, and beef, diet with 1.1 g · kg ⁻¹ · d ⁻¹ , 8 wk | Only protein intake | - | - | - |
| Leenders 2013 (36) | M, F | W+C, 15 g · d ⁻¹ , 24 wk | RT 3x per wk | - | - | - |
| Lockwood 2017 (49) | M | Only W, only Wh, or W+LF; 60 g · d ⁻¹ , 8 wk | RT 4x per wk | ↑→ W; ↑→ Wh; ↑→ W+LF | - | - |
| Mobley 2017 (50) | M | Only W, only Wh, or W+LF; 60 g · d ⁻¹ , 8 wk | RT 4x per wk | ↑→ W+Leu; ↑→ Wh+Leu; ↑→ S+Leu | - | - |

SUPPLEMENTARY TABLE 8 Summary of conclusions from studies investigating the effect of protein intake on strength measurements in a

| Author, year | Sex | Protein intervention (type, amount, duration) | Other interventions | Bench press | Chest press and chest fly | La an ve pu |
|----------------------|------|--|---|-----------------------------|------------------------------------|----------------------|
| Mojtahedi 2011 (5) | F | W, 50 g · d ⁻¹ , 24 wk | AT + ST 2-3x per wk + ER 500 Kcal · d ⁻¹ | - | - | - |
| Nabuco 2018 (51) | F | Wh, 27 g · d ⁻¹ , 12 wk | RT 3x per wk | - | ↑↑ BE; ↑↑ AE | - |
| Naclerio 2017 (61) | M | BF or W, 16.4 or 18 g · d ⁻¹ , 8 wk | RT 3x per wk | ↑→ BF; ↑→ W | - | - |
| Negro 2014 (18) | M, F | Lean beef, 20 g · d ⁻¹ , 8 wk | RT 3x per wk | ↑↗ | - | ↑ |
| Ormsbee 2015 (7) | F | W or C, 30 g · d ⁻¹ , 4 wk | RT + HIIT 3x per wk | - | ↑→ | - |
| Ormsbee 2018 (19) | M, F | Mixed protein shake, 84 g · d ⁻¹ , 24 wk | AT + RT 5x per wk | ↑↑ | - | - |
| Orsatti 2018 (52) | F | S+fat-free milk, 25 g · d ⁻¹ , 16 wk | RT 2-3x per wk | ↑↑ | - | ↑ |
| Ottestad 2017 (38) | M, F | Protein-enriched milk, 40.8 g · d ⁻¹ , 12 wk | Only protein intake | - | ↑→ | - |
| Pihoker 2019 (20) | F | Mixed protein shake, 25 g · d ⁻¹ , 6 wk | RT 2x per wk | ↑→ BE; ↑→ AE | - | - |
| Reidy 2016 (21) | M | W+S+C or W only, 22 g · d ⁻¹ , 12 wk | RT 3x per wk | - | ↑→ | - |
| Rozenek 2002 (62) | M | Protein type not report + milk, 106 g · d ⁻¹ , 8 wk | RT 4x per wk | ↑→ | - | ↑ |
| Schoenfeld 2017 (22) | M | Wh, 25 g · d ⁻¹ , 10 wk | RT 3x per wk | ↑ BE; ↑ AE; timing: → | - | - |
| Seino 2018 (47) | M, F | Protein enriched milk, 10.5 g · d ⁻¹ , 12 wk | RT 2x per wk | - | - | - |
| Snijders 2015 (23) | M | Ch+C, 27.5 g · d ⁻¹ , 12 wk | RT 3x per wk | - | ↑→ | ↑ |
| Taylor 2016 (24) | F | W, 48 g · d ⁻¹ , 8 wk | AgT + ET+ RT 4x per wk | ↑ | - | - |
| Tieland 2012 (40) | M, F | MP, 30 g · d ⁻¹ , 24 wk | Only protein intake | - | - | - |
| Tieland 2012a (41) | M, F | MP, 30 g · d ⁻¹ , 24 wk | RT 2x per wk | - | - | - |
| Vangsoe 2018 (56) | M | Insect protein bars, 0.4 g · kg ⁻¹ , 8 wk | RT 4x per wk | ↑→ | - | - |
| Verdijk 2009 (42) | M | Ch, 20 g · d ⁻¹ , 12 wk | RT 3x per wk | - | - | - |
| Villanueva 2014 (43) | M | W+Cr, 35 g · d ⁻¹ , 12 wk | RT 3x per wk | ↑→ | - | - |
| Volek 2013 (25) | M, F | W or S, ~20 g · d ⁻¹ , 36 wk | RT 3x per wk | ↑→ | - | - |
| Walker 2010 (26) | M | W+Leu, 51.8 g · d ⁻¹ , 8 wk | AT + RT ≥3x per wk | ↑→ | - | - |
| Weisgarber 2012 (27) | M, F | W, 0.3 g · kg ⁻¹ · d ⁻¹ , 8 wk | RT 4x per wk | - | ↑↗ | ↑ |
| Zhu 2015 (60) | F | W, 30 g · d ⁻¹ , 104 wk | Only protein intake | - | - | - |

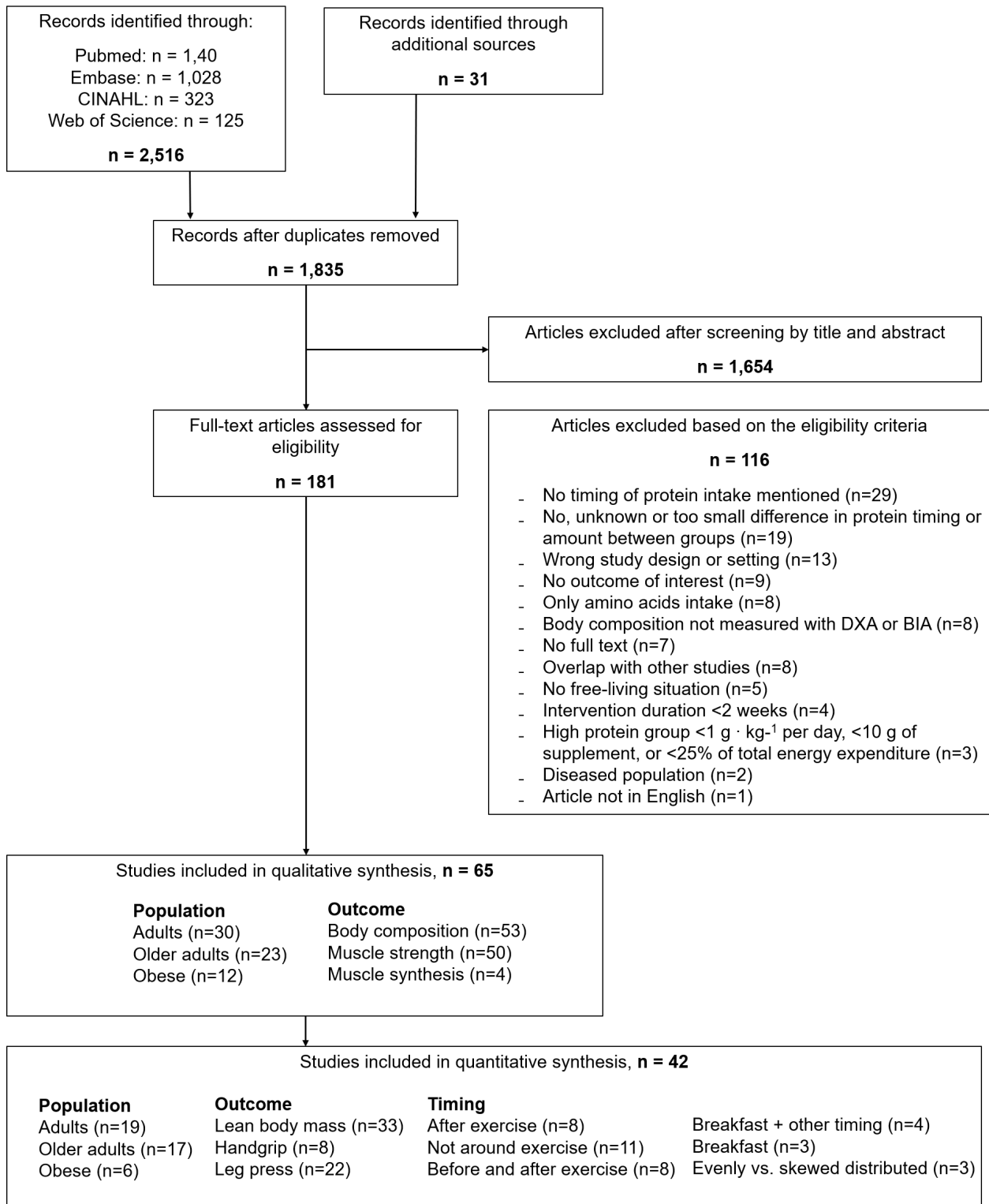
¹ Arrows indicate the direction of change in strength measurements. →, no significant difference; ↑, significant increase; ↗, Increase not significant in protein group, but no significant difference compared with control group; ↑↗, significant increase in protein group and no significant difference compared with control group; AgT, agility training; AT, aerobic training; BE, before exercise; BF, beef protein; BS, before sleep; bW, bioenhanced whey; C, casein; CG, collagen; ED, early in the day; ER, energy restriction; ET, explosive training; EV, evenly; EW, egg white protein; F, female; F-FM, fat-free milk; F-FS, fat-free soy protein; Leu, Leucine; M, male; Mo, morning; MP, milk protein; RISE, resistance + interval + stretching + endurance training; RT, resistance training; S, sports; W, whey protein; Wh, hydrolyzed whey.

² Information obtained from authors.

SUPPLEMENTARY TABLE 9 Results from sensitivity analysis investigating the effect protein intake associated to exercise intervention in older adults

| | | Exercise intervention with protein supplementation (all timings) | Exercise intervention with protein supplementation not consumed around exercise |
|--------------------|----------------------|---|---|
| Lean body mass | Main analyses | 0.58 (0.26 , 0.91) , p = 0.0005 , I ² = 0% No study to be excluded | 0.75 (0.32 , 1.19) , p <0.0001 , I ² = 0% No study to be excluded |
| | Sensitivity Analyses | | |
| Handgrip strength | Main analyses | -0.31 (-1.57 , 0.94) , p = 0.62 , I ² = 61% No study to be excluded | Only one study available (analysis not performed) |
| | Sensitivity Analyses | | |
| Leg press strength | Main analyses | 2.57 (-3.15 , 8.28) , p = 0.38 , I ² = 0% No study to be excluded | 2.94 (-3.76 , 9.63) , p = 0.39 , I ² = 12% No study to be excluded |
| | Sensitivity Analyses | | |
| | | Exercise intervention with protein supplementation consumed at breakfast | Exercise intervention with protein supplementation consumed at breakfast plus another time in the day |
| Handgrip strength | Main Analyses | Only one study available (analysis not performed) | 0.33 (-2.41 , 3.07) , p = 0.81 , I ² = 83% No study to be excluded |
| | Sensitivity Analyses | | |

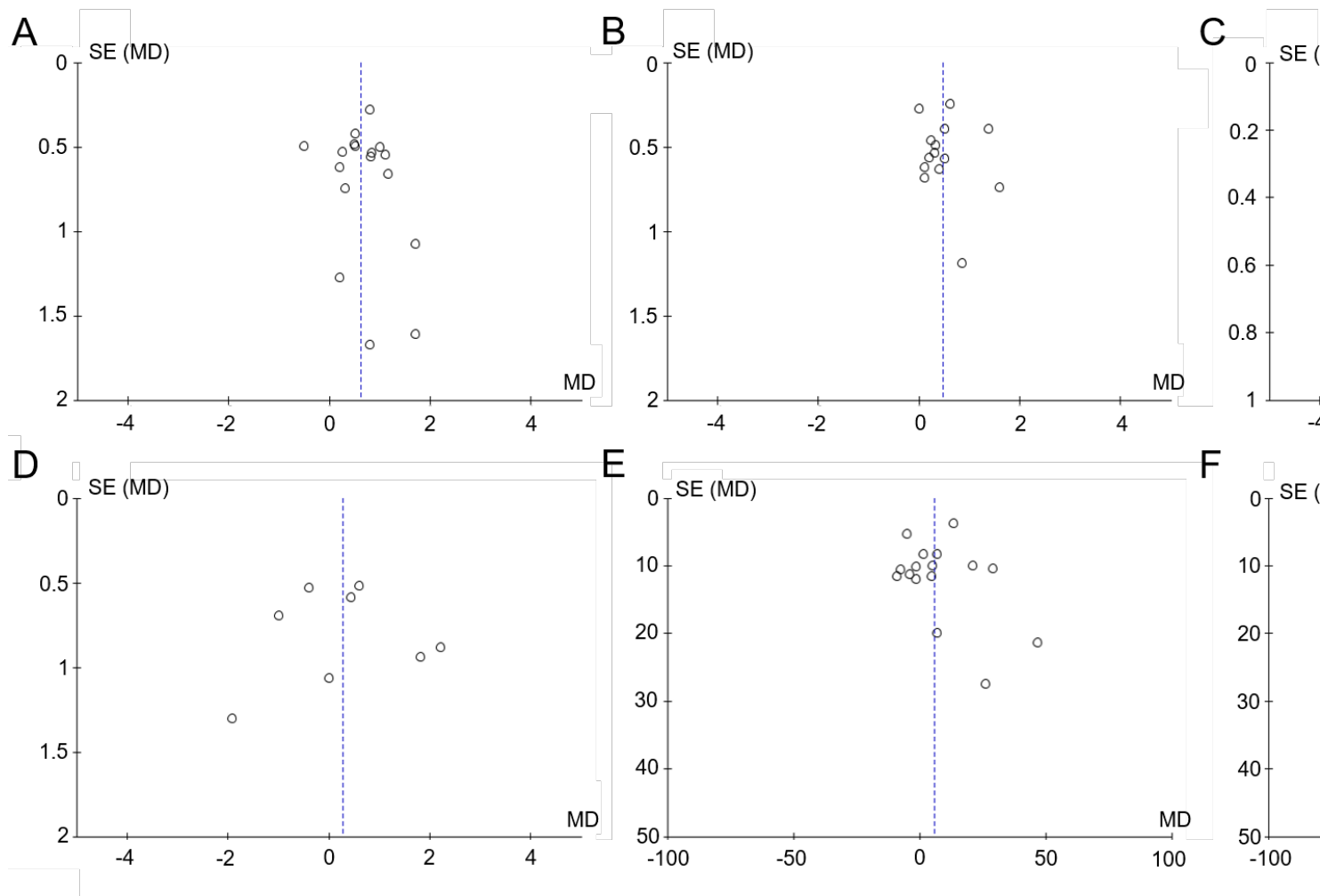
¹ Norton et al. (37) excluded in the sensitivity analysis.



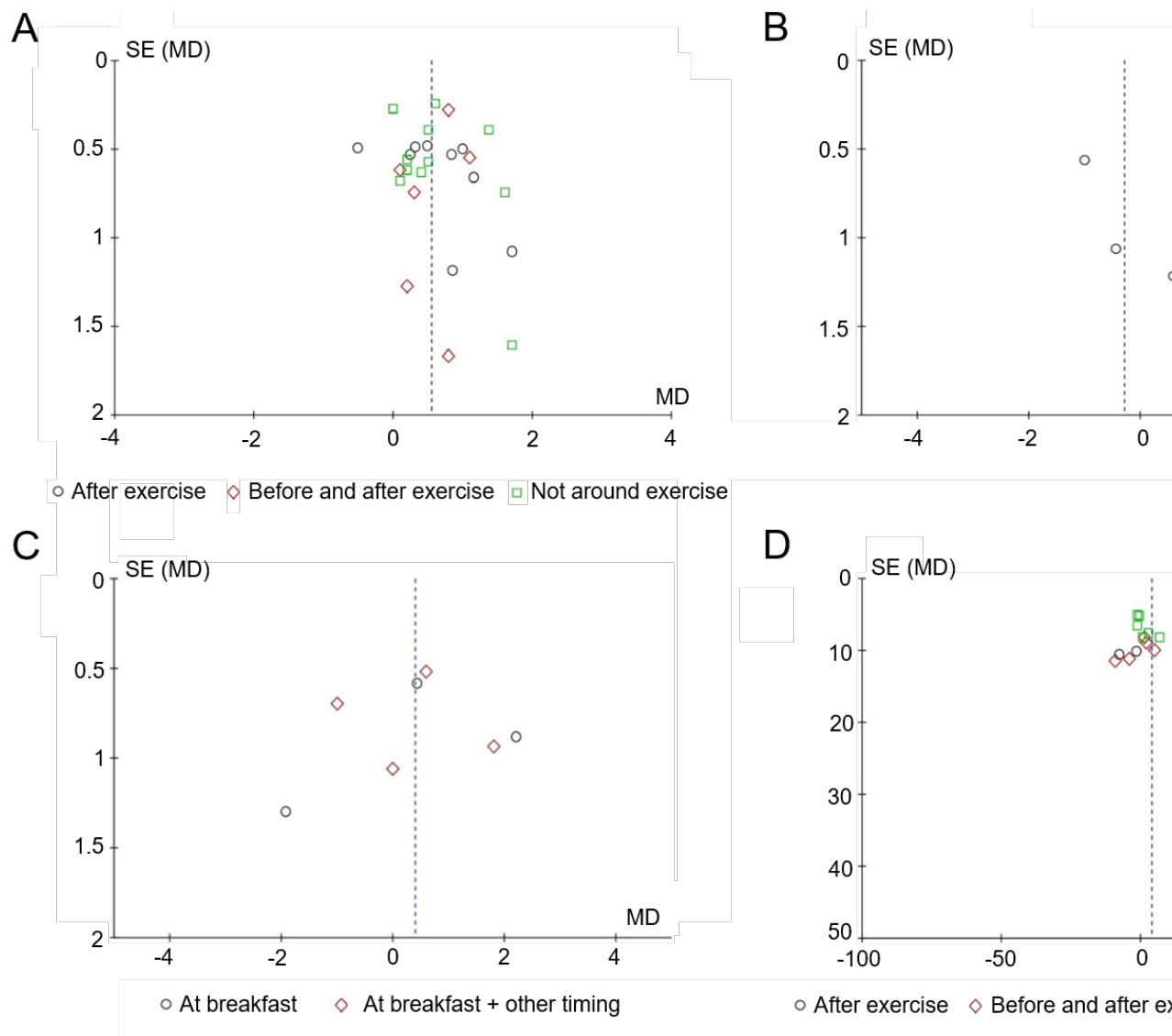
SUPPLEMENTARY FIGURE 1 PRISMA Flow diagram of search and study selection

| | Random sequence generation (selection bias) | Allocation concealment (selection bias) | Blinding of participants and personnel (performance bias) | Blinding of outcome assessment(selection bias) | Incomplete outcome data (attrition data) | Selective reporting (reporting bias) | Other bias |
|----------------------|---|---|---|--|--|--------------------------------------|------------|
| Adechian 2012 (1) | + | ? | ? | + | + | + | + |
| Arciero 2016 (13) | + | + | + | + | + | + | + |
| Baer 2011 (2) | + | ? | + | + | + | + | + |
| Bell 2017 (28) | + | + | + | + | + | + | + |
| Bemben 2010 (54) | + | ? | + | + | + | + | + |
| Brodsky 2004 (64) | + | ? | + | + | + | + | + |
| Burke 2001 (14) | + | + | + | + | + | + | ? |
| Campbell 2018 (63) | + | ? | ? | + | + | + | + |
| Chale 2013 (29) | + | + | + | + | + | + | + |
| Chanet 2017 (30) | + | ? | + | + | + | + | + |
| Coburn 2006 (57) | + | ? | + | + | ? | + | + |
| Daly 2014 (31) | + | + | + | + | + | + | + |
| de Branco 2019 (32) | + | ? | + | + | + | + | + |
| Esmarck 2001 (33) | + | + | + | + | + | + | ? |
| Gryson 2014 (44) | + | + | + | + | + | + | + |
| Hartman 2007 (55) | + | ? | ? | + | + | + | + |
| Herda 2013 (58) | + | + | + | + | ? | + | + |
| Hida 2012 (45) | + | + | + | + | ? | + | + |
| Hoffman 2009 (15) | + | + | + | + | + | + | ? |
| Holm 2008 (34) | + | ? | + | + | + | + | ? |
| Huang 2017 (48) | + | ? | + | + | + | + | + |
| Hudson 2017 (3) | + | + | + | + | + | + | + |
| Hulmi 2009 (59) | + | + | + | + | + | + | + |
| Hwang 2017 (16) | + | ? | + | + | + | + | + |
| Johnston 2017 (4) | + | ? | + | + | + | + | + |
| Josse 2010 (17) | + | + | + | + | + | + | + |
| Joy 2018 (46) | + | ? | + | + | + | + | + |
| Kim 2018 (35) | + | + | + | + | + | + | ? |
| Leenders 2013 (36) | + | ? | + | + | + | + | + |
| Lockwood 2017 (49) | + | + | + | + | + | + | + |
| Mobley 2017 (50) | + | + | + | + | + | + | ? |
| Mojtahedi 2011 (5) | + | + | + | + | + | + | + |
| Murphy 2018 (6) | + | ? | + | + | + | + | + |
| Nabuco 2018 (51) | + | ? | + | + | + | + | + |
| Naclerio 2017 (61) | + | ? | + | + | + | + | + |
| Negro 2014 (18) | + | ? | + | + | + | + | + |
| Norton 2016 (37) | + | ? | + | + | + | + | + |
| Ormsbee 2015 (7) | + | + | + | + | + | + | + |
| Ormsbee 2018 (19) | + | ? | ? | + | + | + | + |
| Orsatti 2018 (52) | + | + | + | + | + | + | + |
| Ottestad 2017 (38) | + | + | + | + | + | + | + |
| Pal 2010 (8) | + | ? | ? | + | + | + | + |
| Pihoker 2019 (20) | + | + | + | + | + | + | + |
| Reidy 2016 (21) | + | + | + | + | + | + | + |
| Robinson 2011 (65) | + | ? | ? | + | + | + | + |
| Rozenek 2002 (62) | + | + | + | + | ? | + | + |
| Schoenfeld 2017 (22) | + | + | + | + | + | + | + |
| Seino 2018 (47) | + | ? | + | + | + | + | + |
| Smith 2018 (9) | + | ? | ? | + | + | + | + |
| Snijders 2015 (23) | + | ? | + | + | + | + | ? |
| Taylor 2015 (24) | + | ? | + | + | + | + | + |
| ten Haaf 2019 (39) | + | ? | + | + | + | + | + |
| Tieland 2012 (40) | + | ? | + | + | + | + | + |
| Tieland 2012a (41) | + | ? | + | + | + | + | + |
| Vangsoe 2018 (56) | + | + | + | + | + | + | + |
| Verdijk 2009 (42) | + | ? | ? | + | + | + | + |
| Verreijen 2014 (10) | + | + | + | + | + | + | + |
| Villanueva 2014 (43) | + | + | + | + | + | + | + |
| Volek 2013 (25) | + | ? | + | + | + | + | + |
| Vorup 2017 (53) | + | + | + | + | + | + | + |
| Walker 2010 (26) | + | ? | + | + | + | + | ? |
| Weisgarber 2012 (27) | + | ? | + | + | + | + | + |
| Wright 2018 (11) | + | + | + | + | + | + | + |
| Wycherley 2012 (12) | + | + | + | + | + | + | + |
| Zhu 2015 (60) | + | + | + | + | + | + | + |

SUPPLEMENTARY FIGURE 2 Summary of risk of bias assessment of the studies included in the systematic review



SUPPLEMENTARY FIGURE 3 Funnel plots of studies investigating the effect of protein intake on the following outcomes: lean body mass in younger adults (n = 696, range of mean age 19.6-42 y) (A), lean body mass in older adults (n = 696, range of mean age 55-81 y) (B), lean body mass in obese adults (n = 696, range of mean age 55-81 y) (C), handgrip strength in older adults (n = 628, range of mean age 67-81 y) (D), leg press strength in older adults (n = 496, range of mean age 55-81 y) (E), and leg press strength in younger adults (n = 395, range of mean age 56.1-81 y) (F). All outcomes are measured in kg. The standard error is plotted on the axis y. The mean difference is plotted on the axis x. Funnel plots were generated using random-effects meta-analyses.



SUPPLEMENTARY FIGURE 4 Funnel plots of studies investigating the effect of timing of protein intake on the following outcomes: lean body mass in obese adults (even vs. skewed protein intake, $n = 104$, range of mean BMI 27.2-33.5 kg/m²; after exercise = 291, range of mean age 20.5-68.1 y; before exercise and after exercise = 127, range of mean age 19.6-72 y; not around exercise = 107, range of mean age 20.5-68.7 y; before exercise and after exercise = 165, range of mean age 20-72 y; not around exercise = 107, range of mean age 20.5-68.7 y) (A), lean body mass in obese adults (even vs. skewed protein intake, $n = 104$, range of mean BMI 27.2-33.5 kg/m²; after exercise = 291, range of mean age 20.5-68.1 y; before exercise and after exercise = 127, range of mean age 19.6-72 y; not around exercise = 107, range of mean age 20.5-68.7 y) (B), breakfast = 273, range of mean age 69-74.3 y; at breakfast plus other timing = 277, range of mean age 67-81 y) (C), and leg exercise = 107, range of mean age 20.5-68.7 y; before exercise and after exercise = 165, range of mean age 20-72 y; not around exercise = 107, range of mean age 20.5-68.7 y) (D). All outcomes are measured in kg. The standard error is plotted on the axis y and the mean difference between groups is plotted on the axis x using random-effects meta-analyses.

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