REVIEW

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Systematic review and meta-analysis of protein intake to support muscle mass and function in healthy adults

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Abstract

We performed a systematic review, meta-analysis, and meta-regression to determine if increasing daily protein ingestion contributes to gaining lean body mass (LBM), muscle strength, and physical/functional test performance in healthy subjects. A protocol for the present study was registered (PROSPERO, CRD42020159001), and a systematic search of Medline, Embase, CINAHL, and Web of Sciences databases was undertaken. Only randomized controlled trials (RCT) where participants increased their daily protein intake and were healthy and non-obese adults were included. Research questions focused on the main effects on the outcomes of interest and subgroup analysis, splitting the studies by participation in a resistance exercise (RE), age (<65 or ≥65 years old), and levels of daily protein ingestion. Three-level random-effects meta-analyses and meta-regressions were conducted on data from 74 RCT. Most of the selected studies tested the effects of additional protein ingestion during RE training. The evidence suggests that increasing daily protein ingestion may enhance gains in LBM in studies enrolling subjects in RE (SMD [standardized mean difference] = 0.22, 95% CI [95% confidence interval] 0.14:0.30, $P < 0.01$, 62 studies, moderate level of evidence). The effect on LBM was significant in subjects ≥65 years old ingesting 1.2–1.59 g of protein/kg/day and for younger subjects (<65 years old) ingesting ≥1.6 g of protein/kg/day submitted to RE. Lower-body strength gain was slightly higher by additional protein ingestion at ≥1.6 g of protein/kg/day during RE training (SMD = 0.40, 95% CI 0.09:0.35, $P < 0.01$, 19 studies, low level of evidence). Bench press strength is slightly increased by ingesting more protein in <65 years old subjects during RE training (SMD = 0.18, 95% CI 0.03:0.33, $P = 0.01$, 32 studies, low level of evidence). The effects of ingesting more protein are unclear when assessing handgrip strength and only marginal for performance in physical function tests. In conclusion, increasing daily protein ingestion results in small additional gains in LBM and lower body muscle strength gains in healthy adults enrolled in resistance exercise training. There is a slight effect on bench press strength and minimal effect performance in physical function tests. The effect on handgrip strength is unclear.

Keywords Muscle mass; Muscle strength; Protein quantity; Physical function

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Introduction

Skeletal muscle is the main component of lean body mass (LBM), and beyond locomotion, muscle has several health-related roles.[1] Reduced skeletal muscle mass and function in adults have been linked to chronic diseases, poor quality of life, sarcopenia, physical disability, increased risk of fractures, and risk for frailty.[2–8] Protein ingestion and resistance exercise (RE) are the main non-pharmacologic factors driving anabolic signals to increase or maintain skeletal muscle mass.[9] Nonetheless, many questions still arise when constructing dietary or physical activity guidelines focusing on skeletal muscle health.[10] Particularly, the optimal daily protein intake level required to optimize skeletal muscle mass gain or maintenance in healthy adults, which is still largely unclear.[10–13] It is also unclear whether additional protein ingestion can preserve lean body mass and muscle function in healthy adults who do not engage in RE.[14]

It appears that ingesting sufficient protein is required to maintain muscle mass.[11,15,16] Recommended protein intakes for healthy adults slightly vary worldwide but are generally in the range of 0.8–0.9 g protein/kg body weight (BW)/day; an intake proposed to maintain nitrogen balance in ~98% of individuals. For example, the current Recommended Dietary Allowance in the USA and Canada is 0.8 g protein/kg BW/day,[17] the FAO of the UN/WHO recommendation is 0.83 g/kg BW/day,[18] and the European Food Safety Authority also established its population reference intake for protein as 0.83 g/kg BW/day for all adults. These dietary protein intake recommendations have traditionally been the same for adults (>18 years old), regardless of age or sex. Nevertheless, a higher daily protein intake (1.2–1.6 g/kg BW/day) has been suggested to improve lean body mass gain or maintain muscle mass in young and old healthy adults.[5,10,19]

Previous meta-analyses have been conducted to ascertain whether additional protein ingestion is linked to increases in LBM (i.e. muscle mass), muscle strength, or physical function in adults.[13,14,20–23] However, the effects of protein ingestion independent of RE are not commonly explored.[13,22] Furthermore, the population inclusion criteria in previous meta-analyses have, we propose, led to confusion in the interpretation of the results. Meta-analyses of studies testing weight loss protocols, including obese subjects or subjects with chronic illnesses, sarcopenia, frailty, chronic diseases, or multigradient supplements, make it challenging to translate the findings.[13,20–22]

We performed a systematic review, meta-analysis, and meta-regression to determine if providing additional dietary protein (protein to exceed participants' habitual protein intake) contributes to increasing LBM (i.e. muscle mass, fat-free mass, lean soft-tissue mass, bone and fat-free mass), strength, and physical/functional test performance in healthy adult subjects who were free from conditions that have been shown to affect skeletal muscle protein turnover and muscle function. Also, we sought to know if additional protein ingestion affects the proposed outcomes independent of RE and age. We hypothesized that additional protein ingestion would improve all outcomes, independent of age or the performance of RE; however, there may be a dose–response effect.

Methods

This systematic review, meta-analysis, and meta-regression followed the guidelines of the Cochrane Handbook for Systematic Reviews of Interventions[24] and the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) report.[25] The procedures for identification, screening, data extraction, and analysis were agreed upon in advance by the team of researchers involved in the study. Details of the intended methods were documented in advance in a protocol registered at the International Prospective Register of Systematic Reviews (PROSPERO, CRD CRD42020159001) before literature search, data extraction, and analysis.

Eligibility criteria

The research questions were established using the PICOS (population, intervention, comparison, outcome, and setting) criteria (*Table* 1). Participants were untrained or trained healthy men or women 18 or older. Studies including weight loss diet protocols, obese, and subjects with any diagnosed or self-reported disease were not included. Studies including obese subjects were excluded since it has been shown that obesity negatively affects postprandial myofibrillar protein synthetic response to nutrition and exercise.[26] Also, interventions were carefully screened for the presence of any potential active ingredient that might impact lean body mass gain other than protein (i.e. creatine, phosphatidic acid, omega-3 fatty acids, anabolic steroids, and beta-hydroxy-beta-methylbutyrate [HMB]). If a particular study testing potential active supplements had intervention groups fitting our inclusion criteria, the study was included and assessed to extract the respective data of protein and control/placebo interventions only. No restrictions were imposed regarding additional protein dose or the duration of the intervention protocol.

Systematic search strategy

A literature search for randomized controlled trials (RCT) investigating the effect of ingesting additional protein on lean body mass, muscle strength, and physical test performance in healthy adults was conducted up to September 2020 by

Table 1 PICOS criteria for inclusion of studies

Parameter	Inclusion criteria	Exclusion criteria
Population	Adult participants (healthy) aged 18 years or older	Subjects with decreased mobility, frailty, obesity, or any chronic or infectious diseases were not included
Intervention	Additional protein ingestion with or without the addition of resistance exercise (increasing protein ingestion as the primary intervention using nutritional supplements or diet at any dose or level)	Intervention aiming to cause weight loss (i.e. negative energy balance) or with the presence of other potential active ingredients in the intervention to change lean body mass (e.g. creatine, phosphatidic acid, omega-3 fatty acids, anabolic steroids, beta-hydroxy-beta-methylbutyrate [HMB])
Comparator	Placebo or no intervention (control)	No control or placebo groups
Outcomes	Lean body mass (or a similar measure), muscle strength (lower body, bench press, and handgrip strength) and performance in physical tests	Not assessing at least one of the target outcomes
Study Design	Randomized controlled trials (RCT)	Not a RCT
Research questions	Main question: • Does a higher level of protein intake increase muscle mass and improve muscle function? Sub-questions: • What is the impact of resistance exercise? • What is the impact of age?	

electronic searching of relevant literature databases. The literature search was conducted on Medical Literature Analysis and Retrieval System Online (Medline), Excerpta Medica (Embase), Cumulative Index of Nursing and Allied Health Literature (CINHAL), and Web of Science core collection. Two distinct search strategies were used to assess studies using protein ingestion only or protein ingestion in parallel to a RE training protocol as an intervention. Results from both searches were combined and screened according to our inclusion and exclusion criteria (see Supplementary files - Search strategies). Limits were applied to the electronic search, restricting studies to adults and humans, English only, and excluding diseases (e.g. cancer and diabetes). The systematic search team (E. A. N., L. C. S., S. R. M., T. Y., and S. M. P.) conducted additional filtering to exclude specific studies based on inclusion and exclusion criteria, visually screening titles, abstracts, and full-texts when necessary. Lists of references from database searches were imported to the software Endnote X9.3.3 for title screening and additional filtering using semi-automated tools. The remaining references selected during title screening were uploaded to Rayyan—a web and mobile app for systematic reviews.[27] Using Ryaan, three reviewers (E. A. N., S. M. P., and T. Y.) screened titles and abstracts independently. Conflicts were solved by reassessing the respective references during group discussion after unblinding the results. Abstracts and conference proceedings were not included.

Data extraction and outcome measures

Studies were reviewed and screened for the study design, protein supplementation or increased protein prescription intervention, subject characteristics, placebo/control information, body composition, resistance training protocols, and strength or physical testing outcomes. Data were extracted not independently by three investigators (E. A. N., S. M. P., and T. Y.) and checked for consistency after extraction. First, each member extracted data from an equal number of different studies. Then, the extracted data were checked for accuracy and reviewed by a second member. Body composition outcomes were extracted as changes in any variable targeting 'muscle' mass (i.e. muscle mass, whole-body lean mass, lean body mass, fat-free mass, and bone and fat-free lean soft tissue mass). Methods applied to measure body composition included dual-energy X-ray absorptiometry (DXA), hydrodensitometry, bioimpedance (BIA), skinfolds, and/or whole-body air plethysmography (BodPod®). Daily protein ingestion, additional protein given during the intervention, protein source (e.g. animal-based, plant-based or blended protein sources) data were also extracted. Strength testing outcomes were repetition-maximum (isotonic) strength (measured by 1-3RM strength tests) or any isometric testing for strength. Upper body strength was obtained from bench/chest press exercises testing data. For lower body strength, leg press, squat, leg extension, leg curls, or similar

exercises were used for data extraction. Physical testing included timed up and go (TUG), chair-based testing, sit-to-stand tests, gait speed tests, balance tests, short physical performance battery tests, stair climb tests, time or distance-limited walking tests, and tests involving activities of daily life. Authors were not contacted for missing data. If not available in tables or the text, data were extracted from figures using the online tool WebPlotDigitizer.[28]

Risk of bias, heterogeneity, quality of the evidence, and sensitivity analysis

The risk of bias was assessed according to the Cochrane Collaboration risk-of-bias tool using RevMan5 by two team members (E. A. N. and S. M. P.).[24,29] Studies were carefully reviewed for details, including randomization methods, participant allocation, and blinding of the subjects and researchers directly involved with the subjects or data analysis. Studies not reporting randomization or blinding procedures were considered high risk in the domain allocation concealment and blinding of participants and personnel. Also, attrition, incomplete outcome data, selective reporting, and other sources of bias were assessed. Cochrane's Q was employed to detect statistical heterogeneity and I^2 statistic to quantify the magnitude of statistical heterogeneity between studies where I^2 30% to 60% represents moderate and I^2 60% to 90% represents substantial heterogeneity across studies. The quality of the evidence was assessed by two team members (E. A. N. and S. M. P.) using the Grading of Recommendations Assessment, Development, and Evaluation (GRADE) system of rating uploading the list of studies on GRADEpro platform (https://gdt.gradepro.org/) and performing the grading manually.[30] Funnel plots were generated for visual assessment for asymmetry and potential publication bias[31] (see *Figures* S20–S29). Studies identified as potential outliers during the visual analysis of funnel plots and assessed with three (3) or more domains judged as potentially high risk of bias were submitted to sensitivity analyses. These analyses were conducted for all outcomes by the 'remove 1' technique to assess whether individual studies had a disproportionate effect on the meta-analyses results[32] (*Table*s S6–S10).

Statistical analysis

The analysis was conducted using change from baseline to immediate post-treatment data (means, standard deviations) for both intervention and control/placebo groups to generate the summary measures of effect in the form of standardized mean difference (SMD). Means and standard deviation (SD) for changes were calculated or imputed from the available data in the paper. Correlation coefficients were estimated from the data and used to impute missing SD for change for some studies according to the methods described in the Cochrane Handbook for Systematic Reviews of Interventions.[24] Calculated correlation coefficients were in the range of 0.7–0.9 for all outcomes. Therefore, a median point of 0.8 was applied as the correlation coefficient for any necessary SD imputation.

The SMD was used as a summary statistic since studies in this systematic review often assessed the same outcome measured in various ways (i.e. muscle mass, lean body mass, bone and fat-free mass and lower body strength measured by leg press, squat, or leg extension). In this situation, it was necessary to standardize the results of the studies before they could be compared across studies or combined in the quantitative synthesis. SMDs were estimated using Hedge's g approach (also known as bias-corrected effect size). The SMDs of 0.2–0.5, 0.5–0.8, and $>$0.8 were considered small, medium, and large effects, respectively. To analyse physical performance measures, we standardized the direction of effect to ensure consistency of desirable outcome responses (i.e. a reduction measured in seconds to cover a given distance reflects a faster gait speed and thus a better outcome, whereas an increase in gait speed measured in m/s reflects a positive outcome). Similarly, a reduction in the sit-to-stand test (s), five chair repetition test (s), and timed up-and-go test (s) is desirable. When available, multiple data were extracted from the same study for lower-body strength or performance in physical tests and included in the analysis.

We used a random-effects three-level meta-analytic approach to account for dependency between effect sizes (i.e. the correlation between effect sizes due to multiple measures or sub-measures of the same outcome within a study or the comparison of multiple interventions to a single control group). In such cases, multiples measures and comparisons from the same study are nested within studies first, and variance in observed effect sizes is decomposed into sampling variance, with-in study variance, and between-study variance to account for intracluster (or intraclass) correlation in the true effects. In addition, we submitted the data to three-level meta-regression analyses based on the use of exercise/resistance training ('yes' or 'no'), age ($<$65 vs. $\geq$65 years old), and the level of protein intake (continuous as g/kg/day or categorical—'$<$1.2 g/kg/day', '1.2–1.59 g/kg/day' and '$\geq$1.6 g/kg/day') when possible. All analyses and figures were made with RStudio v.1.4.1717 (metafor R package).

Results

Literature search and study selection

The results of the literature search are presented in *Figure* 1. Four databases were searched, applying search strategies for

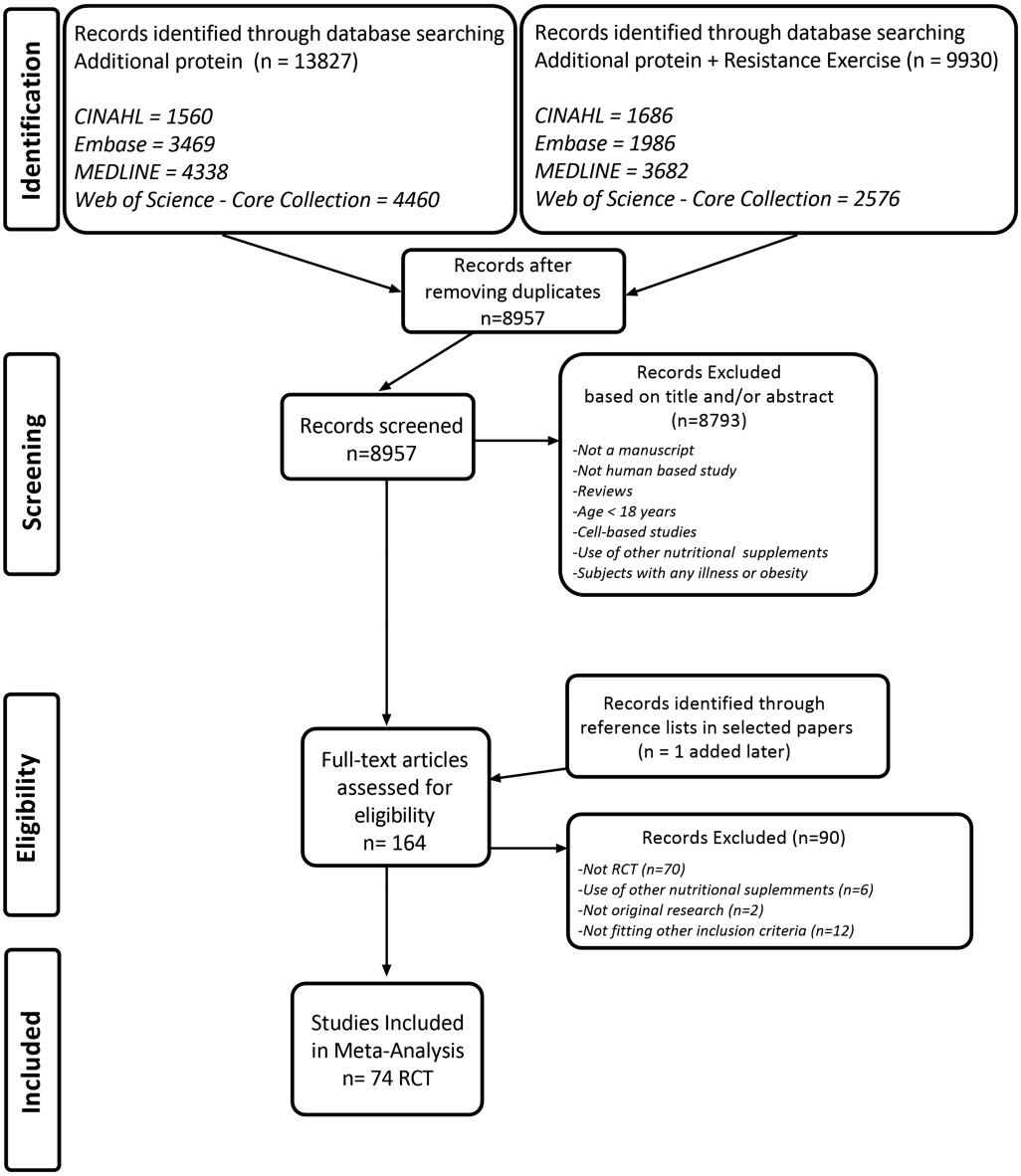

Figure 1 Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) flow chart shows the number of studies involved in each systematic search and screening step. Medline: Medical Literature Analysis and Retrieval System Online.

augmented protein intake alone or in addition to resistance exercise interventions resulting in 23 757 records. After screening for duplicates and study characteristics, 164 studies were selected for full-text screening and eligibility. Finally, 74 RCT were obtained at the end of our screening process.

Study characteristics for randomized controlled trials

Table S1 shows summary information from all RCT studies included in the meta-analysis. In the studies assessing protein ingestion, daily total protein ingestion varied from 1 to 4.4 g/kg/day in the intervention groups (33% of the studies 1.2–1.59 g/kg/day and 54% of the studies ≥1.6 g/kg/day) and from 0.8 to 2.3 g/kg/day in the placebo/control groups. However, it is noteworthy that in ~80% of the studies, baseline protein ingestion was at least 1.2 g of protein/kg/day. The participants' mean age ranged from 19 to 85 years, and study protocols lasted from 6 to 108 weeks (76% of the studies between 8 and 12 weeks). Studies varied vastly regarding the quantity of additional protein provided to research participants. Dietary or supplemented protein ranged from 5 to 100 g/day, depending on the study (56% of the studies between 10 and 30 g/day and 28% between 31 and 50 g/day). Six studies had intervention groups ingesting a blend of

proteins (supplements or food),[33–39] and nine used plant-based (primarily soy) protein supplements.[40–48] In some cases, the same study tested more than one protein source or supplement.[40,42,43,45,47–49]

Sixty-six out of 74 studies were included in the lean body mass change analysis, utilizing 2665 subjects.[33–99] Six studies presented intervention groups not using RE training[41,46,94,97–99]; of these, four studies tested protein ingestion exclusively,[41,97–99] and two studies tested protein ingestion in groups without and with resistance exercise.[46,94] Changes in strength data resulting from the additional protein intervention were extracted from 50 studies testing 2283 subjects for lower-body strength[33,36–39,43,44,47–49,52–58,61–65,67–70,72,74–78,82,84,86,88–92,94,96,100–104] and only three studies with intervention groups without RE.[41,99,105] Thirty-four studies tested bench-press strength[33,36–38,43,47–49,53–55,62–65,67,68,70,72,74,75,77,78,82,84,86–88,90,91,93,95,96,99] with 1049 subjects. The duration of the studies was, on average, 12 weeks for both bench-press and lower body strength. However, one study testing lower-body strength was 108 weeks long.[105] Only one study testing bench-press strength did not use RE in the protocol.[99] Handgrip strength data were extracted from 10 studies in total (612 subjects),[41,50,58,76,81,83,97,99,104,105] four studies using RE training[41,97,99,105] and two studies testing only young participants.[41,81] The approximate duration of the studies testing the effects of protein ingestion on handgrip strength was 12 weeks, except for one study lasting 108 weeks.[105] Data regarding the effects of additional protein ingestion on physical or functional tests were extracted from 15 studies enrolling 1173 subjects[6,39,50,52,56,58,72,75,76,94,97,99,100,104,105] and an approximate duration of 12 weeks,[6,39,50,52,56,58,72,75,76,94,97,99,100,104] except for one study lasting 108 weeks.[105] Eleven studies tested the effect of protein intervention on physical function in parallel to RE training.[39,50,52,56,58,72,75,76,94,100,104]

Risk of bias and heterogeneity of randomized controlled trials

Risk of bias analysis showed that six studies had a potential unclear or high risk of selection bias due to missing information regarding randomization or allocation procedures. Seventeen studies out of 74 presented a potentially high risk of performance bias for blinding research participants or staff. Nine studies reporting the use of single-blind protocols were scored as unclear risk of performance bias. Fourteen studies presented a potentially high risk of detection bias since the research staff was aware of which individuals received the intervention. Eleven studies were scored an unclear risk of detection bias since it was not described whether the research team knew which treatment the participants were assigned to during the intervention and testing. In 21 studies, there was a potential unclear risk of attrition bias. Eleven studies were scored as unclear risk of reporting bias. A summary of the risk of bias analysis is presented in *Figure S1*. A supplementary figure shows the per-study risk of bias analysis (*Figure* S2).

Heterogeneity for overall main effects in most of the analysis regarding changes in lean body mass was low ($I^2 \leq 25\%$) (*Table* 2). Overall heterogeneity was moderate for the main effect of ingesting protein on bench press strength (I^2 = 43%, *Table* 3). Subgroup analysis of studies by age (<65 and ≥65 years old) returned moderate heterogeneity for the overall effect (I^2 = 39.4%, *Table* 3). However, heterogeneity was even higher in the subgroup <65 years old

Table 2 Effects of protein supplementation on changes in lean body mass

Groups/subgroups	SMD	95% CI	Number of trials/ intervention groups	*P*-value	I^2 (%)
All RCT	0.22	0.15:0.29	66/93	<0.01	7
RCT without resistance exercise	0.21	-0.15:0.58	6/6	0.38	25
RCT with resistance exercise (RE)	0.22	0.14:0.30	62/87	<0.01	6.2
<65 years old	0.25	0.16:0.35	48/70	<0.01	8.1
≥65 years old	0.13	−0.00:0.28	14/17	0.06	6.2
RCT with RE reporting protein ingestion	0.19	0.11:0.28	51/72	<0.01	6.9
RCT with RE ingesting <1.2 g/kg/day	−0.14	−0.56:0.27	4/4	0.35	0
RCT with RE ingesting 1.2–1.59 g/kg/day	0.17	0.06:0.28	24/34	<0.01	0
<65 years old	0.15	−0.02:0.31	15/23	0.07	2.8
≥65 years old	0.20	0.02:0.37	9/11	0.03	0
RCT with RE ingesting ≥1.6 g/kg/day	0.30	0.17:0.43	23/34	<0.01	0
<65 years old	0.30	0.17:0.43	23/34	<0.01	0
≥65 years old[a]	-	-	-	-	-
Meta regression – protein ingestion as a continuous variable (g/kg BW/day) in all RCT reporting protein ingestion	0.13	−0.00:0.26	55/77	0.06	NA
Meta regression – protein ingestion as a continuous variable (g/kg BW/day) in studies using RE	0.14	0.00:0.27	51/72	0.04	NA

BW, body weight; CI, confidence intervals; NA, not applicable; RCT, randomized clinical trials; RE, resistance exercise; SMD, standardized mean deviation.
[a]No studies in the dataset.

Table 3 Effects of protein supplementation on changes in bench press strength

Groups/subgroups	SMD	95% CI	Number of trials/ intervention groups	*P*-value	I^2 (%)
All RCT – bench press strength	0.20	0.06:0.34	34/50	<0.01	42.8
RCT without resistance exercise	0.89	−0.07:1.82	1/1	NA	0
RCT with resistance exercise (RE)	0.18	0.04:0.32	33/49	0.01	39.4
<65 years old	0.18	0.03:0.33	32/48	0.01	55
≥65 years old	0.28	−0.51:1.07	1/1	NA	0
RCT with RE testing bench press and reporting protein ingestion	0.15	0.02:0.28	31/46	0.03	27
RCT with RE ingesting <1.2 g/kg/day	−0.16	−1.09:0.77	1/1	NA	0
RCT with RE ingesting 1.2–1.59 g/kg/day	0.17	−0.01:0.35	14/21	0.07	23.3
RCT with RE ingesting ≥1.6 g/kg/day	0.13	−0.15:0.41	16/24	0.33	54.7
Meta regression – protein ingestion as a continuous variable (g/kg BW/day) in all RCT reporting protein ingestion	−0.00	−0.22:0.22	32/48	0.999	NA
Meta regression – protein ingestion as a continuous variable (g/kg BW/day) in studies using RE	0.01	−0.20:0.23	31/47	0.869	NA

BW, body weight; CI, confidence intervals; NA, not applicable; RCT, randomized clinical trials; RE, resistance exercise; SMD, standardized mean deviation.

Table 4 Effects of protein supplementation on changes in lower-body strength

Groups/subgroups	SMD	95% CI	Number of trials/ intervention groups	*P*-value	I^2 (%)
All RCT reporting lower-body strength	0.20	0.08:0.33	50/70	<0.01	52.8
RCT without resistance exercise	0.14	−0.36:0.64	4/4	0.44	20.4
RCT with resistance exercise (RE)	0.21	0.08:0.34	47/66	<0.01	54.5
<65 years old	0.19	0.03:0.36	35/52	0.02	52.8
≥65 years old	0.25	0.01:0.48	12/14	0.04	60.6
RCT with RE reporting protein ingestion	0.21	0.08:0.34	41/56	<0.01	49.5
Ingesting <1.2 g/kg/day	−0.01	−1.85:1.83	2/2	0.95	0
Ingesting 1.2–1.59 g/kg/day	0.08	−0.10:0.27	20/28	0.37	51.6
Ingesting ≥1.6 g/kg/day	0.40	0.23:0.57	19/26	<0.01	26.1
<65 years old	0.38	0.19:0.56	17/24	<0.01	62
≥65 years old	0.55	0.04:1.06	2/2	0.03	0
Meta regression – protein ingestion as a continuous variable (g/kg BW/day) in all RCT reporting protein ingestion	0.25	0.05:0.45	44/60	0.016	NA
Meta regression – protein ingestion as a continuous variable (g/kg BW/day) in studies using RE	0.26	0.05:0.47	41/56	0.014	NA

BW, body weight; CI, confidence intervals; NA, not applicable; RCT, randomized clinical trials; RE, resistance exercise; SMD, standardized mean deviation.

(I^2 = 55%, *Table* 3). When analysed by the level of protein ingestion, overall, and in each protein level subgroup, heterogeneity for bench press strength was small to moderate (I^2 = 23.3–54.7%, *Table* 3). Heterogeneity in low body strength data was moderate (I^2 = 52.8%, *Table* 4). Subgroups of studies by the level of protein intake, resistance exercise presence, and age showed small to high heterogeneity for subgroups (I^2 = 26.1–51.6%, *Table* 4). Handgrip strength data had low heterogeneity (I^2 = 0%, *Table* 5). Heterogeneity in studies reporting physical and functional testing outcomes was moderate (I^2 = 46.4–58%, *Table* 5).

Meta-analysis and meta-regression

Effect of additional protein intake on lean body mass

A summary of the effects of additional protein ingestion on LBM is presented in *Table* 2. Additional protein ingestion probably leads to a small increase in lean body mass (SMD = 0.22, 95% CI 0.15:0.30, $P < 0.01$, n = 66 studies, moderate certainty of evidence) (*Figure* S3). The change represents approximately 1.3–1.4 kg lean mass gain during the intervention compared with an average of ~0.8 kg gain in the placebo/control group (~0.5–0.7 kg difference between groups). We found the same small significant positive main effect on lean body mass gain when isolating studies with resistance exercise (RE) (SMD = 0.22, 95% CI 0.14:0.30, n = 62 studies with RE, moderate certainty of the evidence). Only six studies presented intervention groups assessing LBM when providing additional protein without RE in healthy subjects (*Figure* S3). Our analysis showed a small, non-significant intervention effect when only increased protein ingestion was applied (SMD = 0.21, 95% CI −0.15:0.58, n = 6 studies with intervention groups not using RE, low certainty of the evidence; *Figure* S3). Following this result, we conducted further subgroup analyses only in studies submitting subjects to RE.

Table 5 Effects of protein supplementation on the change of handgrip strength and functional or physical test performance

Groups/subgroups	SMD	95% CI	Number of trials/ intervention groups	*P*-value	I^2 (%)
Handgrip strength – All RCT	0.15	−0.03:0.32	10/11	0.10	0
RCT without resistance exercise	0.20	−0.17:0.57	4/4	0.18	0
RCT with resistance exercise	0.10	−0.18:0.37	6/7	0.43	0
Meta-regression considering protein ingestion as a continuous variable (g/kg BW/day)[a]	−0.09	−1.09:0.91	8/8	0.84	-
Functional and physical performance tests – All RCT[b]	0.15	0.00:0.29	15/19	0.04	46.4
RCT without Resistance Exercise	0.09	−0.08:0.25	5/6	0.28	0
RCT with Resistance Exercise	0.17	−0.03:0.37	11/13	0.10	58
Meta regression – protein ingestion as a continuous variable (g/kg BW/day) in all RCT reporting protein ingestion	−0.23	−0.99:0.52	13/16	0.54	-
Meta regression – protein ingestion as a continuous variable (g/kg BW/day) in studies using RE	−0.26	−1.30:0.77	9/10	0.61	-

BW, body weight; CI, confidence intervals; NA, not applicable; RCT, randomized clinical trials; RE, resistance exercise; SMD, standardized mean deviation.
[a]50% of the RCT accessing handgrip strength reporting protein ingestion were conducted in subjects also submitted to RE.
[b]One study was conducted in subjects <65 years old.

Ingesting additional protein increased LBM in studies with younger subjects and older subjects submitted to RE (SMD = 0.25, 95% CI 0.16:0.35, *n* = 48 studies vs. SMD = 0.13, 95% CI −0.00:0.28, *n* = 14 studies, low certainty of the evidence; *Table* 2). The effect of protein in LBM was more pronounced in young subjects since the main effect in older subjects was marginal and not significant. Still, there was not a significant difference when performing the analysis by subgroups of age ($P > 0.05$) (*Figure* S4). Considering only studies using RE and reporting daily protein ingestion, additional protein still likely has a significant effect on lean body mass (SMD = 0.19, 95% CI 0.11:0.28, $P < 0.01$, *n* = 51 studies, moderate certainty of the evidence; *Table* 2; *Figure* 2). Subgroup analysis by daily protein ingestion showed that ingesting more protein may increase LBM gain in older subjects at 1.2–1.59 g/kg/day (SMD = 0.20, 95% CI 0.02:0.37, *n* = 9 studies, low certainty of the evidence; *Table* 2) and younger subjects at 1.6 g/kg/day or higher (SMD = 0.30, 95% CI 0.17:0.43, *n* = 23 studies, moderate certainty of the evidence; *Table* 2). A *post hoc* sensitivity analysis revealed that excluding Nakayama *et al.*[76] from the subgroup of studies testing older subjects at 1.2–1.59 g/kg/day changed the main effect to a non-significant value (SMD = 0.12, 95% CI −0.08:0.32, *Table* S6). Our systematic search resulted in no studies testing the effect of additional protein ingestion on LBM and RE using intakes ≥1.6 g of protein/kg/day in older subjects (*Table* 2).

A three-level meta-regression considering protein ingestion as a continuous variable in studies using significant but marginal main effect on lean body mass (SMD = 0.14, 95% CI 0.00:0.27, $P < 0.04$, *n* = 51 studies and 72 intervention groups). The same analysis considering all RCT independent of RE returned no significant results (*Table* 2). Bubble plots showing regression curves are in the supplementary files (*Figures* S11 and S12).

Effect of dietary protein intake on muscle strength

Effect of dietary protein intake on bench press strength

Bench press strength gains may be significantly higher in subjects supplemented with protein (SMD = 0.20, 95% CI 0.06:0.34, *n* = 34 studies, low certainty of the evidence) (*Table* 3). Thirty-three out of 34 studies testing the effect of additional protein on bench press strength enrolled subjects in resistance exercise programs (*Figure* S5). When subgrouping studies by the age of subjects, a small positive main effect of additional protein ingestion on bench press strength was detected in <65 years old subjects (SMD = 0.18, 95% CI 0.03:0.33, *n* = 32 studies, low certainty of the evidence). Noteworthy, only one study was conducted on older subjects. The effect was also small on bench press strength considering studies reporting protein ingestion (SMD = 0.15, 95% CI 0.02:0.28, *n* = 31 studies, low certainty of the evidence). Still, no significant effects were found when searching for the effects of different daily protein ingestion levels. However, the sensitivity analysis showed the effect size for the extract of >1.6 g of protein/kg/day changed to significant after excluding Vangsoe *et al.*[91] (*Table* S7). Three-level meta-regression analysis using daily protein ingestion as a continuous variable was not significant for the effect of additional protein in bench press strength (*Table* 3). Bubble plots showing regression curves are in the supporting files (*Figures* S13 and S14).

Effect of dietary protein intake on lower-body strength

Effects of additional protein ingestion on lower-body strength are presented in *Table* 4. Lower-body strength was slightly higher in subjects ingesting more protein (SMD = 0.20, 95% CI 0.08:0.33, *n* = 50 studies, low certainty of the evidence). Four studies measuring lower-body strength did not enrol subjects in RE training, and the effect of ingesting additional protein was not significant (*Figure* S6). However, ingesting more protein produced a small significant effect on

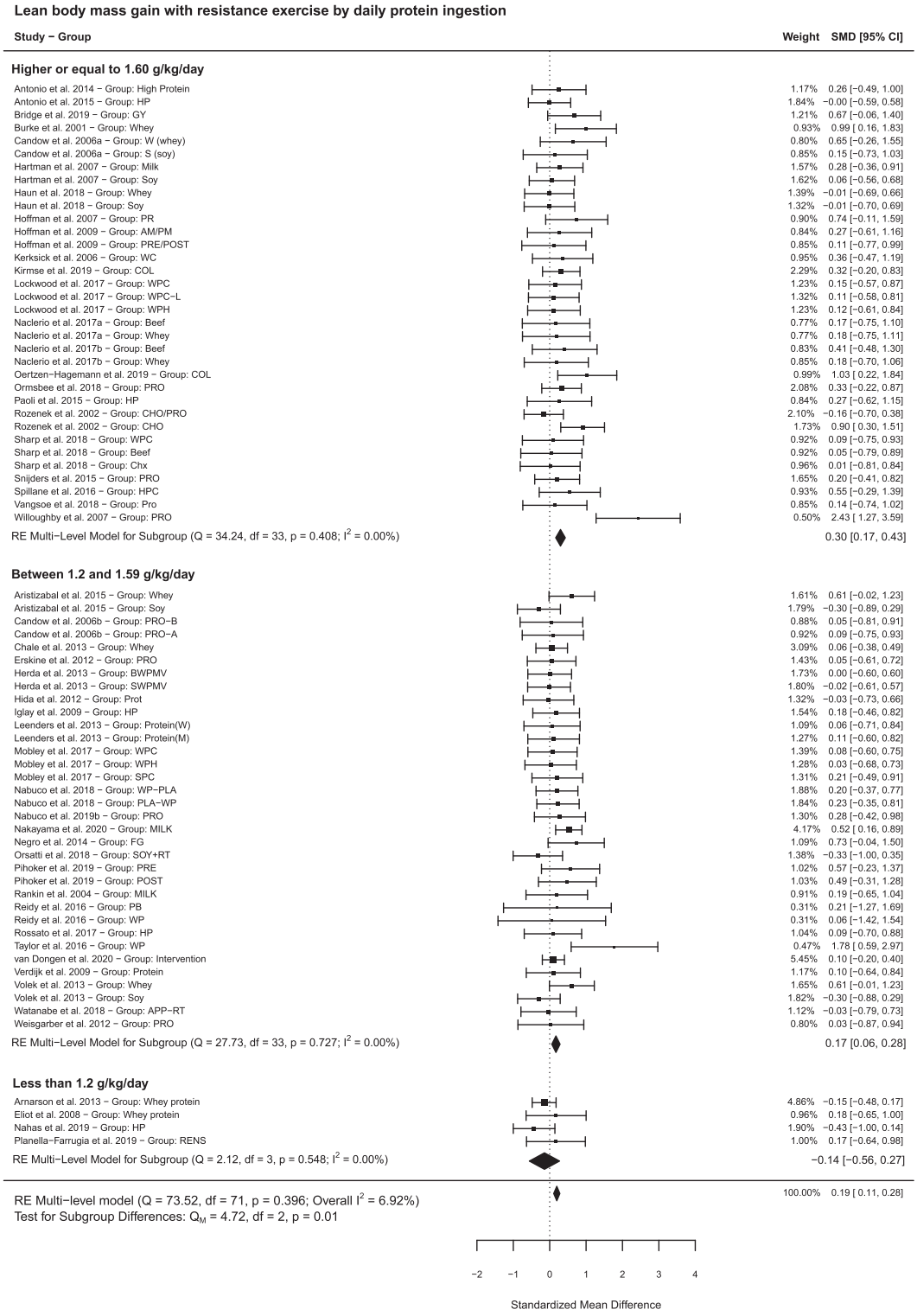

Figure 2 Forest plot showing effects of additional protein ingestion on changes in lean body mass by daily protein ingestion in subjects submitted to resistance exercise training.

lower-body strength in subjects submitted to RE (SMD = 0.21, 95% CI 0.08:0.34, *n* = 47 studies, low certainty of the evidence) (*Figure* S6). This small effect may be independent of the age in subjects submitted to RE (*Table* 4) (*Figure* S7). However, after excluding Burke *et al*. 2001[54] during sensitivity analysis, the significant main effect for older subjects was not

present anymore (SMD = 0.16, 95% CI −0.00:0.32, *Table* S8). Noteworthy, only levels >1.6 g of protein/kg/day may significantly increased lower-body strength (SMD = 0.40, 95% CI 0.23:0.57, *n* = 20 studies, low certainty of the evidence; *Figure* S8) and mainly in young subjects (*Table* 4). Meta-regression using protein ingestion as a continuous variable in studies using RE showed a small significant effect of protein on lower-body strength for all RCT (SMD = 0.25, 95% CI 0.05:0.45, *n* = 45 studies and 60 intervention groups) or studies using RE (SMD = 0.26, 95% CI 0.05:0.47, *n* = 41 studies and 56 intervention groups; *Table* 3). Bubble plots showing regression curves are in the supporting files (*Figures* S15 and S16).

Effect of dietary protein intake on handgrip strength and physical performance functional tests

The evidence shows that ingesting more protein produced a slightly positive but not significant main effect in handgrip strength (SMD = 0.15, 95% CI −0.03:0.32, *n* = 10 studies, very low certainty of the evidence; *Figure* S9). Meta-regression of handgrip strength data vs. protein ingestion as a continuous variable was not significant (*Table* 5 and *Figure* S17).

The evidence was very uncertain about the effect of ingesting more protein and performance on physical and functional performance tests (SMD = 0.15, 95% CI 0.00:0.29, *n* = 15 studies, very low certainty of the evidence). Five studies did not use RE training protocol (*Figure* S10). Meta-regression of performance data in physical and functional tests using daily protein ingestion as a continuous variable was not significant (*Table* 5). Bubble plots showing regression curves are in the supporting files (*Figures* S18 and S19).

Discussion

This systematic review and meta-analysis aimed to investigate the efficacy of increasing dietary protein ingestion to improve lean body mass gain, skeletal muscle strength, and physical function in healthy subjects. To our knowledge, this is the first systematic review and meta-analysis investigating such outcomes restricting the literature search to studies with healthy, not obese adults (i.e. no minor illnesses, not frail, and not sarcopenic), and including no weight-loss study protocols. Furthermore, the literature search was restricted to studies testing protein interventions only (i.e. no additional supplement ingredients). Finally, when considering physical activity intervention, only resistance exercise was in our inclusion criteria. The main findings of the present meta-analysis were that additional protein ingestion together with RE leads to small additional lean body mass and lower body strength. This effect seems to be more prominent in younger subjects ingesting ≥1.6 g/kg/day when enrolled in RE. The number of studies with healthy older subjects to conduct a proper analysis is relatively low what levels down the certainty of the evidence. Effects on bench press strength, handgrip strength, and improved performance in physical tests in healthy adults seem to be trivial. For most outcomes, the evidence is unclear due to the low number of studies or increased heterogeneity. A critical finding of our systematic review is that more RCT testing increasing protein ingestion as a solo intervention in healthy, not obese, adults are needed.

Most meta-analyses have reported consistently positive results regarding the effect of additional protein ingestion on RE training-induced increases in LBM.[13,14,21,23] Cermak *et al*.[23] showed a significant main effect for protein supplementation in muscle mass in young and old subjects during resistance exercise-like training. Tagawa *et al*.[13] found significant effects of additional protein ingestion on LBM in adults (19–81 years old) independent of resistance exercise (≥2 weeks). Wirth *et al*.[21] also found a significant effect of additional protein ingestion on LBM in adults (18–55 years old or ≥55 years old). Conversely, Haaf *et al*.[22] found no effect of additional protein supplementation in LBM in non-frail community-dwelling older adults (>50 years old), even when combined with resistance exercise (≥4 weeks). Noteworthy, is the fact that divergent inclusion criteria are an important source of variability when comparing different meta-analyses. The insertion of clinical trials testing multi-ingredient supplements,[106] including energy-restricted weight-loss diets,[13] or using different cut-off points for age sub-groups likely explain the differences in main effects and conclusions when comparing studies.[14,21–23] Still, a meta-analysis conducted by our group showed that protein ingestion could significantly increase the RE training-induced gains in lean mass in young (<45 years old) and old (≥45 years old) healthy subjects.[14] One of the present meta-analysis objectives was to expand our previous findings to studies that have included protein supplements but not having subjects enrolled in RE training. However, after a systematic review, we identified only six studies matching our criteria, which restricts the possibility of a proper analysis.

Our data show a small increment in LBM caused by ingesting additional protein and RE. Older subjects would likely respond differently since anabolic resistance develops with ageing, and higher per-meal protein doses are postulated to be necessary to stimulate muscle protein synthesis in this population.[107] Present protein ingestion recommendations for healthy young and old subjects range from 0.67 to 0.8 g/kg BW/day.[17,18] This meta-analysis also found that LBM was slightly increased by protein and RE in older subjects in studies testing daily protein ingestion at 1.2–1.59 g/kg/day. However, it is relevant to highlight that the study of Nakayama *et al*.,[76] was the main contributor to this result according to our sensitivity analysis. Probably, because the study sample in Nakayama *et al*.[76] is relatively

large (n = 122) when compared with other studies in the subgroup analysis. Therefore, because the effect of protein supplementation is significant only when Nakayama *et al*.[76] data are included in the analysis, it is possible that resistance exercise *per se* is the main contributor to lean body mass gains in studies with older participants. Curiously, our study showed a significant effect of ingesting more protein and RE in younger subjects only when ingesting ≥1.6 g of protein/kg/day. Our current findings in some way support the hypothesis that higher daily protein ingestion may be needed to increase LBM in young[108] and maybe older healthy subjects.[109] Noteworthy, as highlighted in our results, most of the studies included in our analysis (~80%) reported baseline daily protein ingestion of at least 1.2 g/kg BW. This is 50% higher than current protein ingestion recommendations for healthy adults.[17,18] Such observation might explain the small effect of the intervention on the different outcomes. A relevant question is how much of LBM is muscle mass?[110] This question is relevant as protein supplementation rarely substantially affects strength outcomes,[14,21–23] which highlights that the extra LBM gain stimulated by protein supplementation may not be muscle,[111] or at least not sufficient muscle that is contributing to increases in strength.

According to previous meta-analyses from our group and others, the effects of increasing daily protein ingestion on muscle strength are highly variable.[14,21–23] Previous data from our group[14] and Cermak *et al*.[23] showed very small but significant effect of additional protein ingestion on strength, mainly lower body strength data when selecting RE studies. In contrast, Wirth *et al*.[21] and Haaf *et al*.[22] found no effect of additional protein ingestion and exercise on lower-body strength. However, some particularities in the inclusion criteria in these two meta-analyses (i.e. aerobic exercise training or the cut-off point during age subgroup analysis) might cause such contrast compared with our findings. Our current data support a small effect of ingesting more protein on lower-body strength. Still, a high daily protein ingestion (≥1.6 g/kg BW/day) might be necessary to increase strength in the lower body. Such a level of protein ingestion represents twice the current RDA for protein for healthy adults.[17] This observation reinforces the idea that optimal skeletal muscle increases in strength during RE, while small, might require greater protein ingestion.[14]

Handgrip strength has been positively linked to several relevant variables related to the quality of life and physical function, especially for older subjects.[112] Also, growing evidence shows that handgrip strength is associated with total strength, bone mineral density, fractures, falls, cognitive impairment, depression levels, and overall diet quality.[112,113] However, because few studies investigated the effect of protein ingestion on handgrip strength in healthy adults, it is unclear if additional protein ingestion would improve this outcome. The search strategy used in the present meta-analysis selected 10 studies investigating handgrip strength changes due to additional protein ingestion. Nevertheless, only five studies did not enrol participants in a RE training protocol.

As mentioned, handgrip strength seems to be considered a potential marker related to several aspects of functional capacity and quality of life.[112,113] However, we intended to explore the effects of additional protein ingestion in functional tests directly. We found a small marginal effect of protein ingestion on performance in physical function tests. Our results are in line with a previous meta-analysis[22] showing no significant effect of protein ingestion added to RE on gait speed or chair-rise time in healthy subjects. In contrast, Liao *et al*.[106] found additive significant main effects for additional protein (but included numerous other supplement ingredients) ingestion and RE in the performance of physical function tests in older overweight or obese subjects. Of note, Liao *et al*.[106] compared the effect of additional protein ingestion and RE with no intervention as a control group; therefore, it is reasonable to suggest that the RE was the primary intervention leading to the main findings.

There are several strengths of this review. We restricted our search to studies with healthy non-obese adults. We think this is essential to reduce the influence of minor illnesses such as diagnosed sarcopenia, frailty, arthritis, and even obesity, which have all been shown to perturb muscle protein turnover.[26] Our inclusion criteria excluded studies using multi-ingredient supplements or combining other added nutrients or compounds in the intervention group to isolate the effect of protein. Also, we restricted our systematic search and inclusion criteria to research including resistance exercise only if a study included physical activity. Altogether, these criteria are essential to narrow our findings to the effect of additional protein in healthy adults. Finally, we applied GRADE to qualify our level of evidence. Using GRADE, we show that despite being statistically significant, some of our findings were downgraded in terms of certainty, highlighting that study design issues hamper making further conclusions. The main reasons for downgrading the certainty of evidence were increased risk of bias, mainly in the blinding domains, moderate to high heterogeneity, and, for some subgroup analysis, the low number of the subject in each respective group. In some way, this highlights that future studies testing additional protein as a primary intervention and examining outcomes relevant to strength and lean body mass need to focus on trial planning and control of variables known to affect study quality. We are aware that blinding ingestion in studies testing dietary interventions can be challenging. However, overcoming such challenges might be necessary to increase the quality of the evidence if one is applying the current tools available to grade the evidence in meta-analysis.

We also have some notable limitations that we must acknowledge. In general, study protocols were highly variable,

which is probably the cause of the distinct heterogeneity in response to the intervention. Most of the selected studies in this meta-analysis (65 out of 74) provided animal protein to their subjects. Therefore, our findings reflect mainly the effect of animal-based protein sources.[11] Approximately a quarter of the selected studies showed an increased risk of bias due to poor blinding during the study or the data analysis (*Figures* S1 and S2). The relevance of such increased risk of bias escalates when subgrouping studies by age or levels of daily protein intake. Consequently, some conclusions presented in the current meta-analysis might change in the future in the case of the addition of studies with improved blinding procedures.

In conclusion, our systematic review showed few studies testing protein intervention in healthy non-obese subjects and assessing LMB, strength, or physical function outcomes in the absence of a parallel RE training program. Therefore, more studies are needed to conduct a proper meta-analysis and answer our research question regarding the use of dietary protein intervention solely in healthy subjects. Alternatively, the evidence in this meta-analysis supports the hypothesis that additional protein ingestion (1.6 g of protein/kg/day or higher) leads to small increments in lean body mass in studies enrolling young subjects in RE training. The results on older subjects seem marginal or influenced by individual studies. Lower body muscle strength was also marginally increased by additional protein ingestion in studies with RE training. Bench press strength, handgrip strength, and performance in physical or functional tests were slightly or not affected by ingestion of additional protein. Noteworthy, 80% of the studies reported subjects ingesting at least 1.2 g of protein/kg/day in their habitual diets. Such baseline protein ingestion is a potential contributor for minor or the absence of additional effects of a protein intervention in combination with RE. Still, the downgrading of the evidence for some outcomes in the current meta-analysis highlights the necessity of more studies testing protein interventions in healthy subjects with improved planning of RCTs, fulfilling important aspects as proper blinding of research participants and staff.

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Conflict of interest

Stuart M. Phillips reports that he is an inventor on a patent (WO/2018/157258) held by Exerkine Corporation. Stuart M. Phillips is an unpaid member of the scientific advisory board of Enhanced Recovery™ (https://ersportsdrink.com/). Stuart M. Phillips has received, in the last 5 years, honoraria and travel expenses from the US National Dairy Council, the National Cattlemen's Beef Association, and the US Dairy Export Council. Philip J. Atherton received research funding and/or honoraria for protein nutrition research from Abbott Nutrition, Fresenius-Kabi, and Ajinomoto Co., Inc. Francesco Landi received financial support from Abbott Nutrition and Nutricia. Maria Camprubi Robles, Michelle Braun, and Sandra Naranjo-Modad are employees of Abbott Nutrition, International Flavors & Fragrances, and Givaudan, respectively. Everson A. Nunes, Lauren Colenso-Semple, Sean R. McKellar, Thomas Yau, Muhammad Usman Ali, Donna Fitzpatrick-Lewis, Diana Sherifali, Claire Gaudichon, and Daniel Tomé declare that they have no conflict of interest.

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Online supplementary material

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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