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Protein interventions augment the effect of resistance exercise on appendicular lean mass and handgrip strength in older adults: a systematic review and meta-analysis of randomized controlled trials.

Title

Protein interventions augment the effect of resistance exercise on appendicular lean mass and handgrip strength in older adults: a systematic review and meta-analysis of randomized controlled trials.

Running Head

Protein augments effect of exercise on lean mass

Conflict of Interest Statement

RPK is a beneficiary of a postgraduate stipend from the Institute for Health Research from Liverpool John Moores University. At the time of the analysis of this systematic review, RPK has received a guarantee of support for a planned dietary intervention in the form of food product from Grahams' Family Dairy. RPK has received a speaker honorarium for a symposium hosted by the British Association for Parenteral and Enteral Nutrition and consultation fees from Myprotein. MM, CRG, KL, AJ, TB, FPdH and IGD declare no conflict of interest.

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Abbreviations

ALM: Appendicular lean mass

BIA: Bioimpedance analysis

DXA: Dual energy x-ray absorptiometry

HG: Handgrip strength

KE: Knee extension strength

LBM: Lean body mass

MRI: Magnetic resonance imaging

MPS: Muscle protein synthesis

WMD: Weighted mean difference in change

RE: Resistance exercise

Registry

PROSPERO ID: CRD42019142045

https://www.crd.york.ac.uk/prospero/

Data availability

Data described in the manuscript, code book, and analytic code will be made available upon request pending application and approval.

ABSTRACT

Background: Increased protein intake is suggested as a strategy to slow or reverse the loss of muscle mass and strength observed in sarcopenia, but results from studies that directly tested this possibility have been inconsistent.

Objectives: We assessed the evidence on the effects of whole protein supplementation or higher-protein diets, without the use of amino acids or supplements known to stimulate hypertrophy, alone or in combination with resistance exercise (RE) interventions, on lean body mass (LBM) and strength in older adults.

Design: A systematic search was conducted using PubMed, Medline, Web of Science and Cochrane CENTRAL databases from January 1990 up to July 2021. Randomized controlled trials that assessed the effects of protein supplementation and/or higher-protein dietary interventions in older adults (mean age ≥ 50 years), on total LBM, appendicular lean mass (ALM), handgrip (HG) and knee extension strength (KE) were included.

Results: 28 studies were identified. In pooled analysis, compared with lower protein controls, protein supplementation did not have a significant positive effect on total LBM [weighted mean difference in change (WMD):0.34, 95% CI:-0.21,0.89, I^2:90.01%], ALM [WMD:0.4, 95% CI:-0.01,0.81, I^2:90.38%], HG [WMD:0.69, 95% CI:-0.69,2.06, I^2:94.52%] or KE [WMD:1.88, 95% CI:-0.6,4.35, I^2:95.35%]. However, in interventions that used also RE, statistically significant positive effects of protein were observed for ALM [WMD:0.54, 95% CI:0.03,1.05, I^2:89.76%] and HG [WMD:1.71, 95% CI:0.12,3.30, I^2:88.71%]. Meta-regression revealed no significant association between age, per-meal protein dose, duration, and baseline protein intake with change in any outcome. Sub-group analysis revealed the statistically significant effects on ALM only occurred in sarcopenic/frail populations (WMD:0.88, 95% CI:0.51,1.25, I^2:79.0%). Most studies (n=22) had some risk of bias.

Conclusions: In older adults performing RE, increased protein intake leads to greater ALM and HG, compared with lower protein controls. Without RE, protein has no additional benefit on changes in total LBM, ALM or HG.

Keywords

Protein, resistance exercise, lean body mass, sarcopenia, aging, frailty, strength, elderly

Introduction

By 2050, more than 1 in 5 people, worldwide, will be over 60 years old (1), but while life expectancy is increasing, health span (years free of disease and disability) is not keeping pace (2). A major contributor to poor health and disability in later life is sarcopenia, the age-associated decline in muscle size, strength and quality (3) which accelerates considerably in one's fifties (4). Sarcopenia is positively associated with a great variety of non-communicable diseases including cardiovascular disease and type 2 diabetes mellitus, as well as lower quality of life and mortality (5-7). Decreased muscle strength (dynapenia) precedes a decrease in muscle size (8) and muscle strength is used as a principal determinant of sarcopenia in clinical diagnosis (3, 9).

Reduced activity as we age plays a central role in muscle loss (10, 11), which itself

leads to lower activity levels in older adults (12-14) further compounding muscle loss (15, 16). Additionally, unfavourable hormonal changes (17-19), increases in oxidative stress (20), and inflammation (21-23) all contribute to anabolic resistance (the reduced muscle protein synthesis (MPS) response to anabolic stimuli) (24). As part of the normal aging process, muscle loss is considered primary sarcopenia but when associated with other pathologies, such as diabetes, the muscle loss is considered secondary sarcopenia (25, 26).

Observational studies have identified that higher protein intakes (1.2 *vs.* the recommended 0.8 g/kg body weight/day) may help counteract reduced muscle mass and function associated with aging (27, 28). Dietary protein stimulates muscle protein synthesis and inhibits muscle protein break down, leading to the maintenance or even accretion of lean body mass (LBM) over time (29). This effect is further enhanced when protein is consumed following resistance exercise (RE) (29, 30), thus strengthening the rationale for the benefits of higher protein intakes when combined with exercise.

Alongside whole protein, interventions to augment LBM in older adults may also use

amino acids, vitamins, creatine and essential fatty acids (31). Previous meta-analyses on the effect of protein on LBM have not excluded such substances, rendering it impossible to determine the effect of protein in isolation (32, 33). Therefore an analysis of the effects of RCTs using protein-only interventions, with or without RE is needed. Furthermore, the accrual of LBM is believed to be influenced by numerous factors including per-meal-protein-dose, protein frequency and duration of intervention (34), leading to considerable heterogeneity in interventions aimed at increasing LBM. Therefore, further investigation of the effects of these variables is warranted.

To investigate the role of increased protein in increasing LBM we completed a systematic review and meta-analysis of RCTs assessing the effect of protein supplementation or higher-protein diets, without the use of EAAs or supplements known to stimulate hypertrophy, with or without concomitant RE interventions, on LBM, appendicular lean mass (ALM) and strength in older adults.

Methods

The systematic review protocol was performed in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement guidelines (35). The meta-analysis was carried out following the criteria outlined in the Cochrane Handbook for Systematic Reviews of Interventions (36). The protocol was registered with PROSPERO (ID: CRD42019142045).

Search strategy

PubMed, Medline, Web of Science and Cochrane CENTRAL databases were searched from January 1990 until July 17th, 2021, limiting searches to human RCTs in English language. The following search strategy and keywords were used, as presented, in each database: (diet OR dietary) AND (protein OR whey OR soy OR egg OR casein OR pea) AND (strength OR "lean mass" OR muscle OR "muscle mass" OR hypertrophy OR "body composition") AND (adult* OR "older adult*" OR elder*).

Study selection criteria

Two independent investigators (RPK and CRG) screened titles and abstracts for relevant studies. We included only RCTs that assessed the effects of protein supplementation and/or higher protein dietary interventions in older adults (mean age ≥ 50 years) (4) on LBM or ALM (primary outcome), and if available, strength (secondary outcome) (Table 1). Acceptable measures of LBM were limited to dual-energy X-ray absorptiometry (DXA), bioimpedance analysis (BIA), hydrostatic weighing, air-displacement plethysmography and/or magnetic resonance imaging (MRI). Acceptable measures of strength included handgrip strength (HG) or any reproducible test of 1 repetition maximum (1RM) strength, measured in kilograms. Studies were required to specify duration and only those with a dietary intervention of a minimum of 6 weeks duration were included. Interventions with an energy intake restriction were excluded.

Studies involving supplementation with amino acids, vitamins, performance enhancing drugs and other supplements known to stimulate hypertrophy (such as creatine or *n-3* fatty acids), or studies which did not have at least one intervention group without these substances, were excluded. Studies in populations suffering from pathologies other than

sarcopenia and frailty (*e.g.,* cancer, cardiovascular disease, diabetes etc.) were also excluded.

Data extraction

Two investigators (RPK and CRG) independently extracted data from the original publications. Data on sex, age, health status, baseline protein intake (where available), protein amount, protein source, intervention duration and baseline and endpoint measurements of LBM and/or strength measures were extracted. Where available, data on ALM was extracted. Strength measures for handgrip, knee extension (KE) or leg press were extracted only if absolute measures were available in kilograms. Information on adverse events was also extracted. Any differences in extracted data were resolved by consultation (RPK and CRG) and if necessary, with a third author (MM) until consensus was reached. In order to avoid double counting of control arms, where multiple treatment arms were used with only one control group, priority was given to treatment arms with: RE; dairy proteins; or post-exercise protein. Where data was available in graph form, numerical data was extracted using WebPlotDigitizer (Version 4.3, 2020; https://automeris.io/WebPlotDigitizer). Where necessary data was not

available in the original publication, corresponding authors were contacted and asked to provide said data. Where data was not forthcoming, the article was not included in the specific meta-analysis.

Risk of Bias Assessment

Risk of bias of RCTs was evaluated independently by two investigators (RPK and CRG). The assessment was performed at the study level with the revised Cochrane risk of bias tool which grades the risk of selection, performance, attrition, detection, and reporting biases (37). This tool assesses whether a study has a low, unclear, or high risk of bias. Differences in opinion were resolved by group consultation (RPK, CRG and KL) until consensus was reached.

Statistical analysis

Following the recommendation of the Cochrane Handbook, to calculate the effect size, we used the mean change from baseline to end point in the measures and standard deviation (SD) of the variables of interest for both control and intervention groups (38).

For RCTs, the net changes in measurements (change scores) were calculated as: Net change score = (FT – BT) − (FC – BC) (where FT and BT are the measures at the end of follow-up and at baseline, respectively, in the treatment group, and FC and BC are the corresponding measures in the control group). The net changes in SD of measurements were calculated as: square root $[(SD_{BT})^2 + (SD_{FT})^2 - (2\times R\times SD_{BT}\times SD_{FT})]$ used a correlation coefficient (R) as 0.9 (39) (where SD_{FT} and SD_{BT} are the SD of measures at the end of follow-up and at baseline, respectively, in the treatment group, and SD_{FC} and SD_{BC} are the corresponding SD of the measures in the control group). Studies reported median with interquartile ranges or 95% CIs converted to mean and SD (40). Standard errors (SEs) were converted to SDs using the following formula: SD = SEM × $\sqrt{}$n, where n is the number of participants.

A random-effects model (using the DerSimonian-Laird method) and the generic inverse variance method were used to derive pooled estimates across studies (41). Data were expressed as weighted mean differences (WMDs) with 95 % confidence intervals (CIs). Random effects meta-regression was performed using the unrestricted maximum likelihood method to evaluate the association between exposure and primary outcome

of interest with potential moderators when sufficient data was available. Analyses included pooled analysis of total LBM, ALM, HG and KE along with sub-group analysis of each outcome according to inclusion or exclusion of RE intervention. A further sub-group analysis by health status (healthy or sarcopenic/sarcopenic obese/frail) was performed for the primary outcomes of lean mass (total LBM and ALM). Meta-regression was performed for all outcomes based on baseline outcome measure (Total LBM, ALM, HG and KE, respectively) and, baseline protein intake, per-meal protein dose and intervention duration, and age. Heterogeneity was quantitatively assessed using the I^2 index and Cochrane Q statistic, which measures the extent of true heterogeneity (41). It can be interpreted as the percentage of the total variability in a set of effect sizes due to true heterogeneity, that is, due to between-study variability. Low, moderate, and high I^2 values are 25%, 50%, and 75%, respectively. However, heterogeneity is to be expected in meta-analyses involving different study designs and as such should be quantified with values such as tau-squared (τ^2) (42). Additionally, subgroup analysis according to the exercise status was performed to detect potential sources of heterogeneity. A leave-one-out sensitivity analysis was performed by iteratively removing 1 study at a time to confirm that our findings were not driven by any single study.

We visually inspected the Begg's funnel plot asymmetry and Egger's weighted regression tests to evaluate the potential publication bias when at least 10 studies were involved (43). If publication bias was suspected, this step was followed by adjusting the analysis for the effects of publication bias using the Duval & Tweedie 'trim and fill' methods (43). All analyses were conducted using STATA software, version 16 (StataCorp, College Station, TX). The statistically significant was considered as P values < 0.05.

Results

Flow and characteristics of included studies

Figure 1 shows the flowchart of studies in the review process. After removal of duplicates, 5,680 records were identified by the initial literature search. Through review of titles and abstracts, 99 potentially relevant articles were selected for full-text evaluation. Subsequently, 28 eligible randomized controlled studies met the inclusion criteria (44-71). Due to lack of primary data, 6 of the 28 retrieved papers were not included in the meta-analyses (46, 47, 49, 53, 59, 68) (**Table 2)**.

The characteristics of the studies included in the systematic review are presented in Table 2. Briefly, studies ranged in size from 12 to 196 participants per study, with mean ages of participants ranging from 61 to 85 years. Of the included study populations, 17 were healthy (44, 46-48, 52-55, 57, 58, 60, 62-64, 66, 68, 70) while 6 were considered frail (49-51, 59, 65, 71), 4 were sarcopenic (45, 61, 67, 69) and 1 included participants with sarcopenic obesity (56). Study durations ranged from 10 weeks (3 studies) (55, 58, 60) to 12 weeks (15 studies) (44-46, 48, 53, 56, 57, 59, 61-64, 66, 67, 70), 13 weeks (1 study) (49), 16 weeks (1 study) (52), 24 weeks (7 studies) (47, 50, 51, 54, 65, 69, 71) and 104 weeks (1 study) (68).

Protein interventions

Protein intake was increased in intervention groups using supplementary protein drinks (21 studies) (46, 47, 49-52, 54, 56, 57, 59, 61-71), higher protein diet plans (5 studies) (48, 53, 55, 58, 60) and supplementary protein foods (2 studies) (44, 45). Frequency of supplementary protein intake (excluding studies using high-protein diet plans) ranged from 2 times per week (1 study) (49) to 3 times per week (6 studies) (46, 56, 57, 62, 66,

70), 7 times per week (once daily) (6 studies) (52, 54, 61, 64, 67, 68), 14 times per week (twice daily) (7 studies) (50, 51, 59, 63, 65, 69, 71) and 21 times per week (3 times daily) (3 studies) (44, 45, 47). Per-meal supplementary protein dose also varied with ranges of 5-9 g (5 studies) (44, 45, 47, 49, 69), 10-19 g (8 studies) (51, 52, 54, 63, 65-67, 71), 20-29 g (6 studies) (46, 50, 59-62, 64) and ≥30 g (5 studies) (56, 57, 61, 68, 70). Sources of supplementary protein included whey protein (10 studies) (46, 47, 50, 52, 56, 57, 62, 68-70), mixed milk protein (10 studies) (49, 51, 52, 54, 59, 63-65, 68, 71), ricotta cheese (2 studies) (44, 45), soy protein (1 study) (61), casein (1 study) (66) and collagen (1 study) (67).

Exercise interventions

Of the 28 articles included in this review, 19 made use of RE in at least one arm of their intervention (46, 48-54, 56-58, 60-62, 64, 66, 67, 70, 71). The frequency of RE was relatively consistent, ranging from twice per week (5 studies) (49, 51, 61, 70, 71) to 3 times per week (14 studies) (46, 48, 50, 52-54, 56-58, 60, 62, 64, 66, 67). The RE involved numerous different protocols including resistance machines only (9 studies) (46, 48, 50, 51, 53, 54, 64, 66, 71), machines and free-weights (8 studies) (52, 56-58,

60, 62, 67, 70), elastic resistance bands (61) (1 study) and high intensity functional exercise (49) (1 study). The number of repetitions used in all but one study ranged from 6 to15. The remaining study, which used elastic resistance bands (61), did not provide data on the number of repetitions used. All 19 studies which made use of RE incorporated some form of progressive resistance *i.e.,* the intensity, resistance, or volume of the exercises performed were increased over the course of the intervention period.

Outcome measures

The majority of articles included measured body composition using DXA (23 studies) (44-46, 50-60, 62-69, 71) with the remaining articles using BIA (4 studies) (47, 49, 61, 70) and hydrostatic weighing (1 study) (48). Data was extracted for LBM and ALM, where available. Strength and muscle function measures varied greatly amongst the included studies and two strength measures were selected for meta-analysis due to their frequency of use and the availability of data: handgrip (10 studies) (44, 45, 51, 55, 61, 63-65, 69, 71); and 1 RM knee extension (8 studies) (51, 56-58, 62, 65, 66, 71).

Amongst the 28 studies included in the systematic review, only 13 reported on whether intention-to-treat (ITT) or per-protocol (PP) analysis was used (44, 45, 49, 50, 55, 58, 63-65, 67-69, 71). Of these, 9 studies published data from intention-to-treat (ITT) analyses (44, 45, 49, 50, 58, 65, 68, 69, 71) and 4 published data from per-protocol (PP) analyses (55, 63, 64, 67). As only one set of data was available from each of the studies (ITT or PP), no particular set of data was prioritized in the data extraction for our study. Five studies (44, 50, 64, 65, 68) completed both analyses, but published results from only one, and in all cases, it was specified that the results were similar in both analyses.

Adverse Events

Information on adverse events, where available (8 studies) (44, 45, 50, 55, 63, 64, 67, 68), is reported in **Supplementary Table 1**.

Risk of Bias Assessment

Risk of bias of RCTs was evaluated with the revised Cochrane risk of bias tool. This tool

determined 6 studies had low risk of bias (47, 50, 51, 65, 67, 71), 18 studies had some concerns of bias (44, 45, 48, 49, 52-55, 57, 59, 60, 62-64, 66, 68-70) and 4 studies had high risk of bias (46, 56, 58, 61) (**Supplementary Figure 1**). Regarding dietary protocol adherence, only 11 studies provided details on how this was monitored and included: collection of used supplement containers (50, 59, 61, 63, 65, 67-69); observation by research staff (71); adherence phone calls from research staff (58); and dietary counselling with provision of key foods (64).

Meta-analysis

Total lean body mass

A pooled estimate of the effect of protein on total LBM using 21 intervention groups involving 967 participants revealed the change in total LBM was not statistically significantly different between the protein intervention and lower protein control groups (weighted mean difference in change (WMD): 0.34, 95% CI: -0.21, 0.89, I^2: 90.01%) (**Figure 2**). Sub-group analysis of those interventions that did not use a RE arm (7 intervention groups) revealed that additional protein did not result in a change in total LBM compared to the lower protein control group (WMD: 0.18, 95% CI: -0.14, 0.51, I^2:

0.00%) (Figure 2). In interventions that did use RE (14 interventions), sub-group analysis revealed the change in LBM was not statistically significantly greater in protein interventions compared with lower protein control groups (WMD: 0.29, 95% CI: -0.45, 1.04, I^2: 93.33%) (Figure 2). Results of tests for heterogeneity for no-RE group, RE group, between group and overall were p=0.78, p<0.001, p=0.79 and p<0.001, respectively.

Meta-regression analysis revealed that changes in total LBM were not significantly associated with any of the tested mediators including: baseline total LBM (β = 0.04, 95% CI: 0.0, 0.08, ρ = 0.054, $I^2_{residual}$ = 77.47%); age (β = 0.07, 95% CI: -0.01, 0.14, ρ = 0.1, $I^2_{residual}$ = 75.93%); per-meal protein dose (β = -0.05, 95% CI: -0.11, 0.02, ρ = 0.13, $I^2_{residual}$ = 64.78%); intervention duration (β = 0.07, 95% CI: 0.0, 0.13, ρ = 0.06, $I^2_{residual}$ = 74.47%); baseline protein intake (β = 3.21, 95% CI: -0.53, 6.94, ρ = 0.09, $I^2_{residual}$ = 81.68%); and frequency of protein intervention (β = 0.04, 95% CI: -0.04, 0.12, ρ = 0.28, $I^2_{residual}$ = 63.87%).

Pooled sub-group analysis of studies by health status revealed that neither healthy nor

unhealthy (sarcopenic, sarcopenic obese & frail) populations experienced greater increases in total lean body mass compared with lower protein control groups (n=11, WMD: 0.23, 95% CI: -0.49,0.96, p=0.53 and n=10, WMD: 0.5, 95% CI: -0.17,1.17, p=0.15, respectively).

A sensitivity analysis was performed, comparing RCTs that used double blinded, placebo-controlled methodology with those that did not. Sensitivity analysis revealed there was no significant difference in change in total LBM between protein groups and lower protein control groups in double blinded nor non-double blinded studies (n=12, WMD: 0.42, 95% CI: -0.31,1.16, p=0.26 and n=9, WMD: 0.16, 95% CI: -0.26,0.58, p=0.45, respectively).

Appendicular lean mass

A pooled estimate of the effect of protein on ALM using 10 intervention groups involving 470 participants revealed the change in ALM was not statistically significantly different between the protein intervention and lower protein control groups (WMD: 0.4, 95% CI: -0.01, 0.81, I^2: 90.38%) (**Figure 3**). Sub-group analysis of those interventions that did not

use a RE arm revealed that additional protein did not result in a change in ALM compared to the lower protein control group (WMD: 0.05, 95% CI: -0.12, 0.21, I^2: 0.00%) (Figure 3). However, in interventions that did use RE, sub-group analysis revealed the change in ALM was statistically significantly greater in protein interventions compared with lower protein control groups (WMD: 0.54, 95% CI: 0.03, 1.05, I^2: 89.76%) (Figure 3). Results of tests for heterogeneity for no-RE group, RE group, between group and overall were p=0.61, p<0.001, p=0.07 and p<0.001, respectively.

Sub-group analysis of studies by health status and use of RE revealed that only unhealthy (sarcopenic, sarcopenic obese & frail) populations that performed RE experienced greater increases in total lean body mass compared with lower protein control groups (WMD: 0.88, 95% CI: 0.51, 1.25, I^2: 79.0%). No effect was observed in frail populations without RE (WMD: 0.3, 95% CI: -0.13, 0.2, I^2: 0.0%) nor healthy populations with or without RE (WMD: -0.08, 95% CI: -0.96, 0.80, I^2: 75.0% and WMD: 0.26, 95% CI: -0.42, 0.95, I^2: 0.0%, respectively).

Meta-regression analysis revealed that changes in ALM were not significantly

associated with any of the tested mediators including: baseline ALM (β = -0.36, 95% CI: -0.16, 0.09, ρ = 0.52, $I^2_{residual}$ = 86.88%); age (β = 0.05, 95% CI: -0.02, 0.11, ρ = 0.16, $I^2_{residual}$ = 83.36%); per-meal protein dose (β = 0.01, 95% CI: -0.03, 0.05, ρ = 0.59, $I^2_{residual}$ = 88.91%); intervention duration (β = 0.03, 95% CI: -0.03, 0.1, ρ = 0.33, $I^2_{residual}$ = 85.23%); and baseline protein intake (β = 1.7, 95% CI: -18.1, 21.49, ρ = 0.8, $I^2_{residual}$ = 91.88%).

Handgrip strength

A pooled estimate of the effect of protein on HG using 11 intervention groups involving 629 participants revealed the change in HG was not statistically significantly different between the protein intervention and lower protein control groups (WMD: 0.69, 95% CI: -0.69, 2.06, I^2: 94.52%). Sub-group analysis of those interventions that did not use a RE arm revealed that additional protein did not result in a change in HG compared to the lower protein control group (WMD: -0.01, 95% CI: -0.39, 0.38, I^2: 0.00%) (Figure 4). However, in interventions that did use RE, sub-group analysis revealed the change in HG was statistically significantly greater in protein interventions compared with lower protein control groups (WMD: 1.71, 95% CI: 0.12, 3.3, I^2: 88.71%) (**Figure 4**). Results of

tests for heterogeneity for no-RE group, RE group, between group and overall were p=0.55, p<0.001, p=0.04 and p<0.001, respectively.

Sub-group analysis of studies by health status and use of RE revealed that only unhealthy (sarcopenic, sarcopenic obese & frail) populations that performed RE experienced greater increases in HG compared with lower protein control groups (WMD: 2.06, 95% CI: 0.66, 3.47, I^2: 84.3%). No effect was observed in frail populations without RE (WMD: 0.0, 95% CI: -0.41, 0.41, I^2: 0.0%) nor healthy populations with (n=1) or without RE (WMD: -1.0, 95% CI: -3.35, 1.35 and WMD: 0.1, 95% CI: -1.59, 1.79, I^2: 42.99%, respectively).

Meta-regression analysis revealed that changes in HG were not significantly associated with any of the tested mediators including: baseline handgrip strength (β = 0.02, 95% CI: -0.13, 0.17, ρ = 0.79, $I^2_{residual}$ = 91.62%); age (β = 0.19, 95% CI: -0.02, 0.39, ρ = 0.07, $I^2_{residual}$ = 87.04%); per-meal protein dose (β = -0.04, 95% CI: -0.26, 0.18, ρ = 0.69, $I^2_{residual}$ = 92.68%); intervention duration (β = 0.12, 95% CI: -0.07, 0.31, ρ = 0.19, $I^2_{residual}$ = 88.99%); and baseline protein intake (β = 14.36, 95% CI: -10.75, 39.47, ρ =

0.19, $I^2_{residual}$ = 93.88%).

Knee extension strength

A pooled analysis of 8 intervention groups involving 335 participants revealed the change in KE was not statistically significantly different between the protein intervention and lower protein control groups (WMD: 1.88, 95% CI: -0.6, 4.35, I^2: 95.35%) (**Figure 5**). Sub-group analysis by use of RE revealed that in the RE sub-group, KE was not statistically significantly greater in protein interventions compared with lower protein control groups (WMD: 1.37, 95% CI: -1.01, 3.76, I^2: 93.06%) (**Figure 5**). Only one study not including RE, which used frail participants, was available for analysis. This study reported the change in KE was statistically significantly greater in protein interventions compared with lower protein control groups (WMD: 5.0, 95% CI: 3.91, 6.09). Results of tests for heterogeneity for RE group, between group and overall were p<0.001, p<0.001, p=0.007 and p<0.001, respectively.

Meta-regression analysis revealed that changes in knee extension strength were not significantly associated with: baseline knee extension strength (β = 0.16, 95% CI: -0.09,

0.4, ρ = 0.17, $I^2_{residual}$ = 93.49%), age (β = 0.21, 95% CI: -0.31, 0.73, ρ = 0.35, $I^2_{residual}$ = 93.19%); per-meal protein dose (β = -0.11, 95% CI: -0.5, 0.29, ρ = 0.52, $I^2_{residual}$ = 93.53%); and intervention duration (β = 0.04, 95% CI: -0.48, 0.55, ρ = 0.87, $I^2_{residual}$ = 94.3%). However, a trend was observed for an association with baseline protein intake (β = 23.61, 95% CI: -0.47, 47.68, ρ = 0.053, $I^2_{residual}$ = 89.59%). A summary diagram of the main results from these meta-analyses can be seen in **Figure 6**.

Sensitivity analysis

In leave-1-out sensitivity analyses, the pooled effect estimates remained similar across all studies and their subgroups, which confirmed that the statistically significant difference between the studied groups is the overall effect of all included studies.

Publication bias

No evidence for funnel plot asymmetry was found, and Eggers test showed no evidence of small study effect for LBM (p=0.969), ALM (p=0.863), HG (p=0.767), or KE (p=0.985)

Discussion

In the present study, we systematically reviewed RCTs investigating the effect of increased protein intake on muscle mass and strength, with or without exercise interventions, in older adults. To our knowledge, this is the first meta-analysis to show that whole protein interventions, without the use of EAAs or supplements known to stimulate hypertrophy, lead to superior gains in appendicular lean mass and handgrip strength in frail older adults, only when combined with an RE intervention.

Analysis of all applicable studies revealed that protein interventions increased ALM and HG but only in interventions that included an RE component. This highlights that the benefits of RE on ALM accrual and HG are augmented by higher protein intakes in older adults. As such, RE interventions to improve ALM and strength in the elderly may benefit from protein supplementation. This increase in ALM may be of clinical significance as Brown *et al.* (72), using data from older adults (mean age 74.9 y) participating in the Third National Health and Nutrition Examination Survey, 1988–1994, observed that each 5.5 kg increase in ALM was robustly associated with a 50% lower risk of mortality [HR: 0.5 (95% CI: 0.27,0.92); p=0.03]. The combination of protein with

resistance exercise was determined in this meta-analysis to result in an increase in ALM (WMD: 0.54, 95% CI: 0.03, 1.05, I^2: 89.76%) which may be viewed as clinically important.

The results of our analysis are partially in agreement with previous meta-analyses by Hou *et al*. (32) and Liao *et al.* (73), which investigated the effects of protein or amino acid supplementation together with RE on muscle mass and physical function. Hou *et al*. (32) reported that protein increased fat-free mass, appendicular skeletal muscle mass, HG, KE and leg press strength, while Liao *et al.* (73) reported greater lean mass and leg strength gains. One key metric in which our results differ with the meta-analyses by Hou *et al.* and Liao *et al.* (32, 73) is that we did not observe an increase in KE. While sub-group analysis by use of RE revealed no positive effect of protein in the RE sub-group, only one study not including RE, which used frail participants, was available for analysis. This study reported the change in KE was statistically significantly greater in protein interventions compared with lower protein control groups (WMD: 5.0, 95% CI: 3.91, 6.09). The reason for this result in a study which did not incorporate RE is not clear and as this result from one single study does not constitute a meta-analysis, it

should not be considered representative of similar interventions.

However, our results contrast with meta-analyses by Tieland *et al*. (33) and Ten Haaf *et al*. (74) which found no additional improvements in LBM or strength with increased protein. A potential reason for this discrepancy is that both meta-analyses included studies that used EAAs for the intervention, whereas our meta-analysis only included studies which used whole protein. The ingestion of whole protein (whey) has been observed to result in greater skeletal muscle protein accrual than ingesting the equivalent content of constituent EAAs alone (75). However, when whey (15 g) is compared with an isocaloric quantity of EAA (15 g), the EAA-induced rate of MPS is greater, although other studies have reported that similar doses of EAA (15 g) have not resulted in increased muscle mass after 24 weeks (76). It is possible that the inclusion of multiple studies using EAAs with overall low amounts of protein (≤ 15 g per dose) may have led to the non-statistically significant results of the findings of the aforementioned papers. For example, of 6 studies which used supplementary amino acids in the meta-analysis by Ten Haaf *et al.,* only 2 reported improvements in LBM (77, 78) with no statistically significant improvements in total LBM reported in the remaining

4 studies (76, 79-81).

It is also important to highlight that the meta-analysis by Tieland *et al*. (33) did not include studies that used exercise interventions, which augment the MPS-stimulating effect of acute protein ingestion (29). As our meta-analysis revealed that protein-induced improvements in ALM were only observed in interventions which included RE, it is reasonable that a meta-analysis of studies without RE would show no benefit of added protein. A further difference from our study is that the meta-analysis by Ten Haaf *et al*. (74) only used interventions in non-frail, community-dwelling older adults. One might speculate that those suffering from frailty may have lower muscle mass than healthy older adults and might be more likely to benefit from interventions aimed at increasing muscle mass. Indeed, our sub-group analysis revealed that sarcopenic/frail populations performing RE did experience significant increases in ALM. As such, the results of our study lend support to the concept that populations at greatest risk of muscle and strength loss may increase ALM through RE with protein supplementation.

Anabolic resistance to protein ingestion is one potential explanation for the lack of effect

of protein intervention in some of the studies included in our systematic review (24). Twenty grams of protein may be sufficient to maximally stimulate MPS in young people (82, 83) however, bolus doses of 40 g of protein have been shown to stimulate the MPS response more robustly in older adults (84). As such, larger per-meal doses of total protein (30-40 g/meal) may be useful for stimulating MPS in older populations (85, 86). In our review, of the 25 studies included, only six used interventions involving per-meal-protein-boluses of 30 g or more (56, 57, 60, 61, 68, 70). Therefore, the majority of studies included in our systematic review may have been using protein doses which sub-maximally stimulate MPS and lean mass accrual. However, our meta-regression revealed that higher per-meal protein doses were not associated with greater increases in total LBM or ALM accrual.

The anabolic action of protein intake may be especially relevant in the post-exercised state, as the MPS response to the presence of amino acids is known to be augmented after a bout of RE (86) for more than 24 hours post-exercise (87). Therefore, frequent stimulation of MPS via protein ingestion in this anabolically sensitive period may further benefit the accrual of muscle mass (88). This may partially explain why we only

detected a statistically significant effect of protein supplementation on ALM in interventions using RE.

Data on baseline protein intake was only available for 17 of the included studies. In these studies, the average protein intake was 0.91 g/kg of body weight per day, which is higher than the protein reference nutrient intake (RNI) of 0.75 g/kg/d (89). It may be speculated that many of the populations included in our meta-analysis might not benefit from further protein supplementation, as would be expected in those with lower baseline protein intakes below the RNI (34). While our meta-regression revealed that baseline protein intake tended to be positively associated with increases in KE, the effect was not statistically significant (p=0.053). Further information on baseline protein intake would have allowed for a more thorough analysis of its specific effects on LBM accrual.

Our meta-analysis revealed that the effect of protein on HG strength was only statistically significant in interventions that used RE, specifically in frail/sarcopenic populations. Handgrip strength is frequently used as an indicator of strength, physical function and health in older adults (90-93) and is also used as part of the diagnostic

criteria for sarcopenia itself (3). However, research by Tieland *et al*. indicates that handgrip strength may not be an ideal outcome measure to evaluate the efficacy of RE interventions in elderly individuals (94). In contrast to our results, Tieland *et al*. (65) observed no difference in handgrip strength between intervention and placebo groups, despite improvements in leg muscle strength and physical performance in the intervention group. One explanation for this is that the strength and size adaptations of muscle to RE are specific to the muscle trained (95). As handgrip-specific training is not a frequent modality in RE programs, improvements in handgrip strength may not be expected. As such, our finding supports the use of handgrip strength as a useful measure of efficacy of interventions aimed at improving lean mass and strength.

A particular strength of our study is that it includes meta-analyses of both total LBM and ALM. Animal studies have shown that high protein intakes can result in visceral organ hypertrophy, which can contribute to increases in total LBM (96). As such, ALM may be a more appropriate measure of skeletal muscle hypertrophy, and thus the efficacy of protein and exercise interventions, than total LBM. We may speculate that increases in ALM, rather than total LBM, are more desirable for improving muscle strength and

function in older people. Indeed, appendicular skeletal muscle mass (a specific measure of ALM) is used as part of the diagnostic criteria for sarcopenia, as specified by the European Working Group on Sarcopenia in Older People (EWGSOP) (3). In our analysis, only ALM was found to be positively influenced by protein intake but only in frail/sarcopenic populations and only when combined with RE (not without) and may be more clinically significant than total LBM.

There are also some limitations to this study. Firstly, this was an aggregate data analysis as opposed to an individual participant data (IPD) analysis due to poor response to requests for IPD from authors. IPD analysis can overcome some issues of aggregate analysis such as selective reporting, publication bias and low power to detect interactions at the individual level (97). Secondly, and in line with this first point, it was not possible to investigate sex-specific effects of protein or RE in these studies, which may be of interest due to potential differences in MPS between sexes (98).

Conclusion

In conclusion, compared with lower protein controls, protein supplementation leads to

increases in appendicular lean mass and handgrip strength in older adults, but only when combined with resistance exercise. With 22 of 28 studies presenting some risk of bias, caution should be exercised in the interpretation of these results.

Conflict of Interest Statement

RPK is a beneficiary of a postgraduate stipend from the Institute for Health Research from Liverpool John Moores University. At the time of the analysis of this systematic review, RPK has received a guarantee of support for a planned dietary intervention in the form of food product from Grahams' Family Dairy. RPK has received a speaker honorarium for a symposium hosted by the British Association for Parenteral and Enteral Nutrition and consultation fees from Myprotein. MM, CRG, KL, AJ, TB, FPdH and IGD declare no conflict of interest.

Author's Contributions

RPK, CRG, KL, FPdH, IGD, TB designed the review; RPK and CRG conducted the systematic review and extracted data; MM and AJ performed the data analysis; RPK and MM wrote the paper; RPK had primary responsibility for final content. All authors read and approved the final manuscript.

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Table 1

Inclusion and exclusion criteria

Inclusion Criteria	Exclusion Criteria
Population	**Population**
・Mean age >50 years ・Male and/or female ・Healthy, frail or sarcopenic	・Individuals with pathologies including cardiovascular disease, type 2 diabetes, cancer, cachexia, chronic kidney disease, immunodeficiency disease etc
Intervention	**Intervention**
・Randomized controlled trial ・Supplementary protein, high-protein food or high-protein dietary intervention ・Non-supplemented control or protein intake lower than intervention group ・With or without resistance exercise ・Minimum duration of 6 weeks	・Isolated amino acids ・Anabolic steroids, hormones, vitamins or supplements known to induce hypertrophy
Primary outcomes	
・Lean body mass or fat-free mass (kg) ・Appendicular lean mass or skeletal muscle mass (kg) ・Measured using dual-energy X-ray absorptiometry (DXA), bioimpedance analysis (BIA), hydrostatic weighing, air-displacement plethysmography and/or magnetic resonance imaging (MRI)	
Secondary outcomes	
・Hand grip strength or 1-repetition maximum strength test (kg)	
Other	**Other**
・Full paper ・English language	・Protocol papers ・Abstract only

Table 2. Participant characteristics and intervention details of the 25 included studies.

Author		n	Mean age (years)	Health Status	Baseline protein intake (g/kg/d)[1]	Intervention/ Control	Frequency of protein intervention (per week)	Added protein dose (g/dose)	Total added protein (g/day)	Resistance Exercise Protocol	Duration (weeks)	Main finding, Intervention *vs* Control	Included in meta-analysis
Aleman-Mateo *et al* (2012) (45)	Intervention	20	76	Sarcopenic	N/A	Ricotta cheese	21	5.2	16	No	12	ALM, LBM in arms and muscle strength ↑ in men only	Yes
	Control	20			N/A	Habitual diet							
Aleman-Mateo *et al* (2014) (44)	Intervention	50	70.2	Healthy	N/A	Ricotta cheese	21	6	18	No	12	ALM and physical performance ↑	Yes
	Control	50			N/A	Habitual diet							
Arnarson *et al* (2013) (46)	Intervention	75	74	Healthy	1.0 ± 0.3	Whey protein	3	20	20	Yes	12	No greater gains in lean mass, strength, or physical function	No
	Control	66			0.9 ± 0.3	CHO[2]							
Bjorkman *et al* (2012) (47)	Intervention	46	83.6	Healthy	N/A	Whey protein in juice	21	6.7	20	No	24	Body weight ↑ and maintenance of skeletal muscle mass	No
	Control	51			N/A	Juice							
Campbell *et al* (1994) (48)	Intervention	6	65	Healthy	N/A	Higher protein diet plan	N/A	N/A	63	Yes	12	No greater increase in LBM	Yes
	Control	6			N/A	Lower protein diet plan							
Carlsson *et al* (2011) (49)	Intervention	89	84.5	Frail	N/A	Milk protein	2.5	7.4	7	Yes	13	No greater increase in LBM	No
	Control	88			N/A	Placebo							
Chale *et al* (2013) (50)	Intervention	42	77.7	Frail	0.91[3]	Whey protein	14	20	40	Yes	24	No greater increases in LBM, strength, power, or physical function	Yes
	Control	38			0.93[3]	Isocaloric control							
Dirks *et al* (2017) (51)	Intervention	17	76.5	Frail	N/A	Milk protein	14	15	30	Yes	24	Type I and type II muscle fiber hypertrophy ↑	Yes
	Control	17			N/A	Placebo							
Gryson *et al* (2014) (52)	Intervention	27	60.8	Healthy	N/A	Milk protein or whey protein	7	10	10	Yes	16	Muscle mass and strength ↑ and muscle fatigue ↓	Yes
	Control	18			N/A	Placebo							
Iglay *et al* (2009) (53)	Intervention	18	61	Healthy	N/A	Higher protein diet plan	N/A		6	Yes	12	No greater increase in LBM	No
	Control	18			N/A	Lower protein diet plan							
Leenders *et al* (2013) (54)	Intervention	27	70	Healthy	1.2[3]	Milk protein	7	15	15	Yes	24	No greater increases in LBM, strength, or functional capacity	Yes
	Control	26			1.2[3]	CHO placebo							
Li *et al* (2021) (69)	Intervention	31	71	Sarcopenic	N/A	Whey protein	14	7.9	15.8	No	24	No greater gains in LBM, ALM or grip strength	Yes
	Control	30			N/A	Habitual diet							
Mitchell *et al* (2017) (55)	Intervention	15	74.2	Healthy	1.1 ± 0.3	Higher protein diet plan	N/A	N/A	48	No	10	LBM and knee-extension power output ↑	Yes
	Control	16			1.2 ± 0.4	Lower protein diet plan							
Nabuco *et al* (2018) (57)	Intervention	43	66.7	Healthy	0.93 ± 0.36	Whey protein	3	35	35	Yes	12	ALM, muscular strength, and functional capacity ↑	Yes
	Control	23			0.97 ± 0.28	CHO placebo							
Nabuco *et al* (2019 A) (56)	Intervention	13	69.1	Sarcopenic obese	0.93[3]	Whey protein	3	35	35	Yes	12	ALM ↑ and trunk fat mass ↓	Yes
	Control	13			0.95 ± 0.27	CHO placebo							
Nabuco *et al* (2019 B) (70)	Intervention	15	69.2	Healthy	0.94 ±0.3	Whey protein	3	35	35	Yes	12	LBM ↑ and waist circumference and body fat ↓	Yes
	Control	15			0.94 ±0.3	CHO placebo							

Nahas *et al* (2019) (58)	Intervention	22	63.4	Healthy	0.76 ± 0.05	Higher protein diet plan	N/A		23	Yes	10	Functional capacity but no additional increase in strength and LBM ↑	Yes
	Control	25			0.76 ± 0.06	Lower protein diet plan							
Ottestad *et al* (2016) (59)	Intervention	17	77	Frail	1.0 ± 0.3	Milk protein	14	20	40	No	12	No greater increase in LBM or strength	No
	Control	19			1.0 ± 0.3	Isocaloric control							
Rossato *et al* (2017) (60)	Intervention	11	63.2	Healthy	0.79[3]	Higher protein diet plan	N/A	20-30	24	Yes	10	No greater increase in LBM	Yes
	Control	12			0.75[3]	Lower protein diet plan							
Shahar *et al* (2013) (61)	Intervention	30	67.1	Sarcopenic	0.83[3]	Soy protein	7	20 (M), 40 (W)	20 (M), 40 (W)	Yes	12	Upper body strength ↑ but no greater increase in LBM	Yes
	Control	35			0.91[3]	Habitual diet							
Sugihara Junior *et al* (2018) (62)	Intervention	15	67.6	Healthy	0.85 ± 0.1	Whey protein	3	27	35	Yes	12	ALM and strength ↑	Yes
	Control	16			0.81 ± 0.1	Isocaloric CHO control							
ten Haaf *et al* (2019) (63)	Intervention	58	69	Healthy	0.86 ± 0.23	Milk protein	14	15	31	No	12	LBM ↑ and fat mass ↓	Yes
	Control	56			0.92 ± 0.24	Isocaloric control							
Thomson *et al* (2016) (64)	Intervention	118	61.5	Healthy	N/A	Dairy protein or soy protein	7	27	27	Yes	12	No greater increases in LBM, strength, or physical function	Yes
	Control	61			N/A	Habitual diet							
Tieland *et al* (2012 A) (65)	Intervention	34	79.5	Frail	1.0 ± 0.0	Milk protein	14	15	30	No	24	Physical performance ↑ no greater increase in ALM	Yes
	Control	31			1.0 ± 0.0	Placebo							
Tieland *et al* (2012 B) (71)	Intervention	31	78	Frail	1.0 ± 0.1	Milk protein	14	15	30	Yes	24	LBM and ALM ↑, no greater increase in strength	Yes
	Control	31			1.0 ± 0.1	Placebo							
Verdijk *et al* (2009) (66)	Intervention	13	72	Healthy	1.1 ± 0.1	Casein protein	3	10 (pre-exercise), 10 (post-exercise)	20	Yes	12	No greater increase in LBM or strength	Yes
	Control	13			1.1 ± 0.1	Placebo							
Zdzieblik *et al* (2015) (67)	Intervention	26	74.3	Sarcopenic	N/A	Collagen protein	7	15	15	Yes	12	LBM and strength ↑ fat mass ↓	Yes
	Control	27			N/A	Silica placebo							
Zhu *et al* (2015) (68)	Intervention	101	74.3	Healthy	1.2 ± 0.3	Milk plus whey protein	7	30	30	No	104	No greater increase in LBM or physical function	No
	Control	95			1.1 ± 0.3	Isocaloric skim milk placebo							

[1]Mean ± SD where available, or calculated means from available data; [2]CHO: carbohydrate; [3]Value calculated from available data; M: men; W: women.

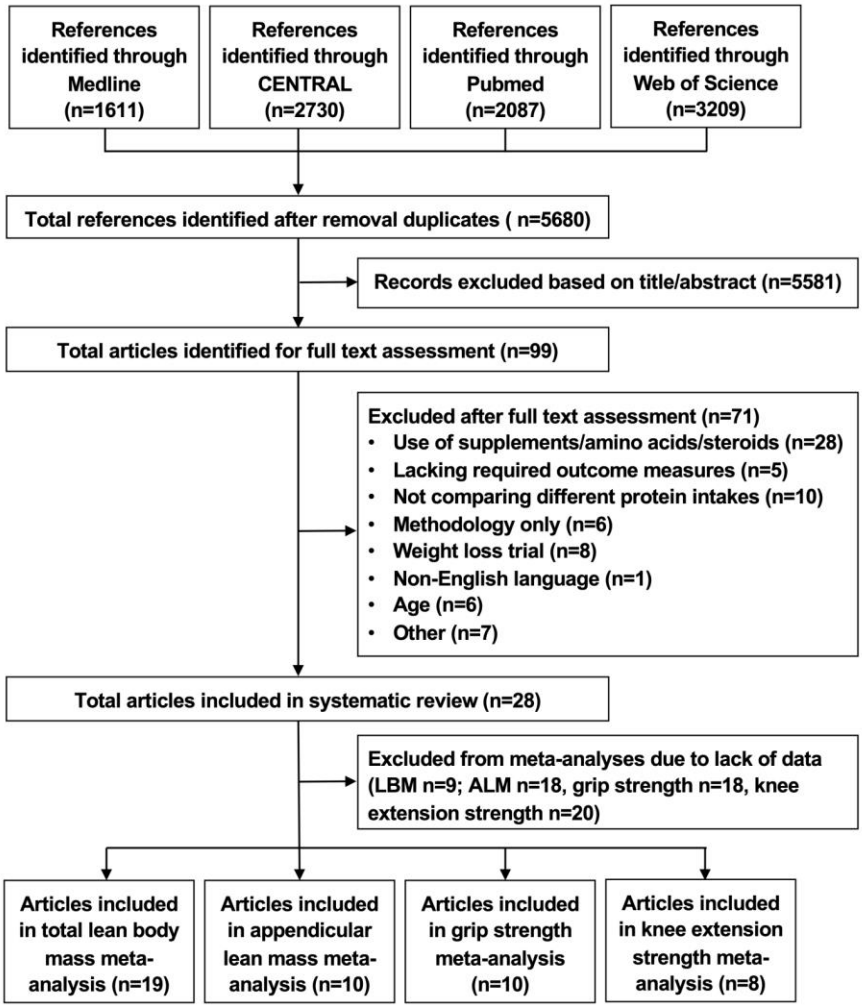

FIGURE 1 *PRISMA flow chart of studies through systematic review process. PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analysis.*

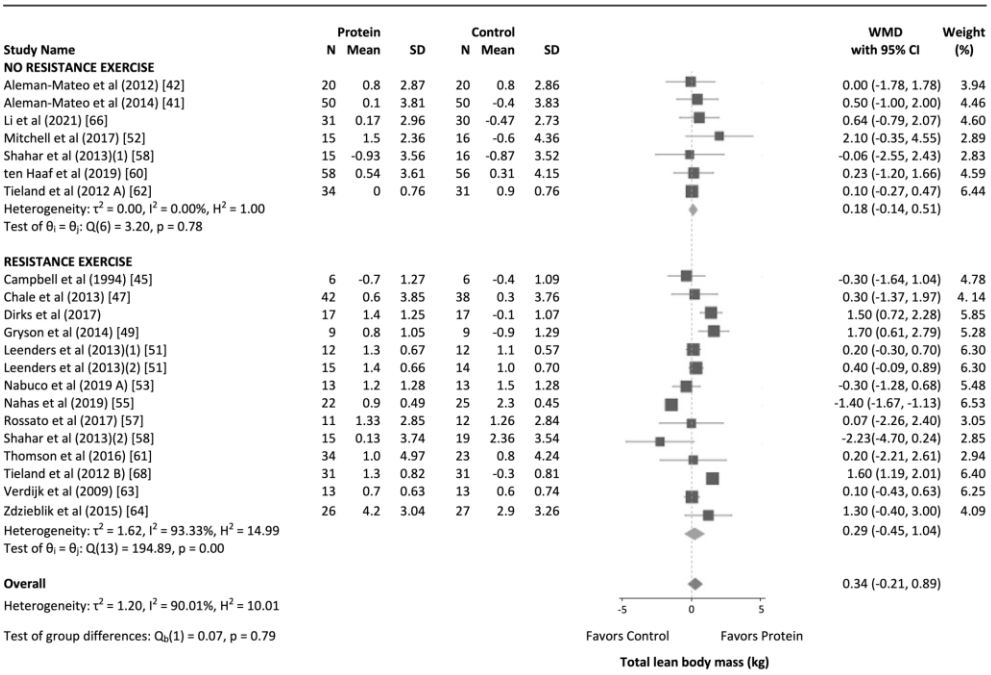

FIGURE 2 Forest plot of standard difference between lower protein control and protein groups on lean body mass of 21 intervention arms organized by trials that did not, and did, use RE. A random effects model (using the DerSimonian-Laird method) and the generic inverse variance method were used to derive pooled estimates across studies. Squares indicate the point estimate for each trial, with the size of the square proportional to the contribution of the study to the overall estimate. The overall estimate and 95% confidence interval are indicated by the diamonds. Where two versions of the same study are mentioned, results from two different intervention arms were reported:

*Shahar 2013 (61): (1) no resistance exercise, (2) resistance exercise; Leenders 2013 (54): (1) females, (2) m*ales. *Nabuco (2019 A (56) & B (70)) and Tieland (2012 A (65) & B (71)) are different studies published in the same year.*

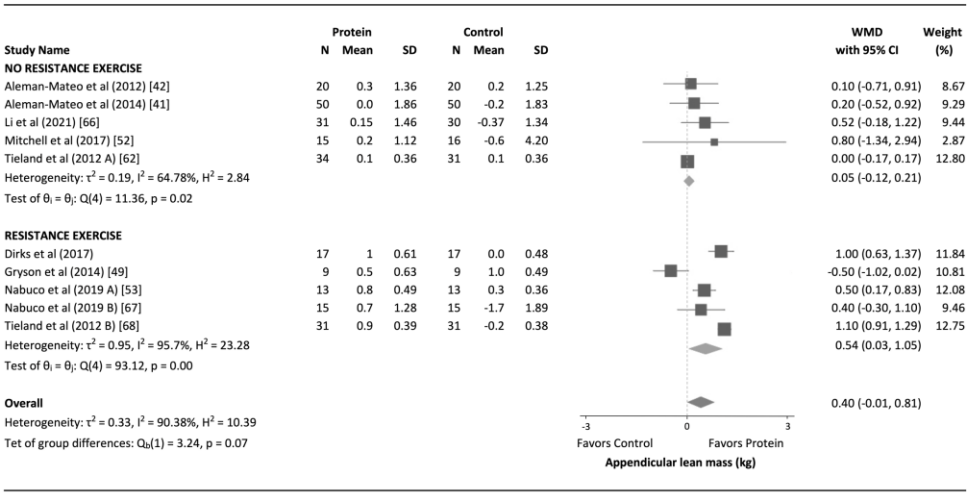

FIGURE 3 Forest plot of standard difference between control and protein groups on appendicular lean mass of 10 intervention arms organized by trials that did not, and did, use RE. A random effects model (using the DerSimonian-Laird method) and the generic inverse variance method were used to derive pooled estimates across studies. Squares indicate the point estimate for each trial, with the size of the square proportional to the contribution of the study to the overall estimate. The overall estimate and 95% confidence interval are indicated by the diamonds. Nabuco (2019 A (56) & B (70)) and Tieland (2012 A (65) & B (71)) are different studies published in the same year

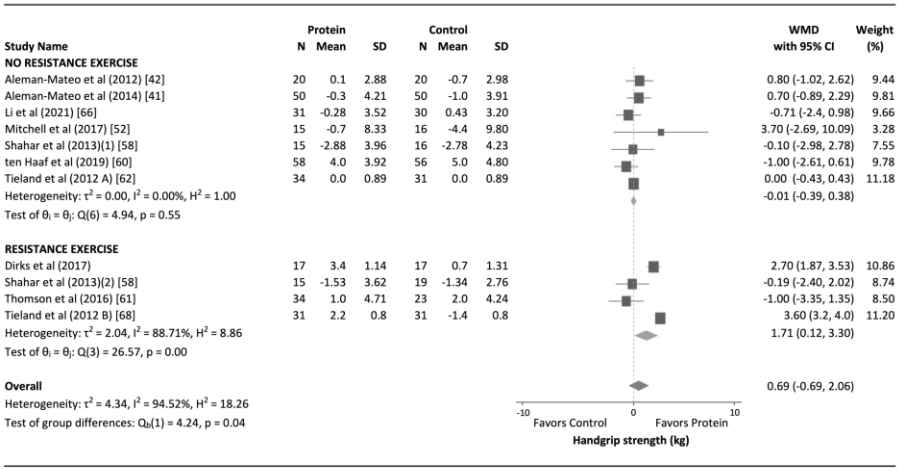

FIGURE 4 Forest plot of standard difference between control and protein groups on handgrip strength of 11 intervention arms organized by trials that did not, and did, use RE. A random effects model (using the DerSimonian-Laird method) and the generic inverse variance method were used to derive pooled estimates across studies. Squares indicate the point estimate for each trial, with the size of the square proportional to the contribution of the study to the overall estimate. The overall estimate and 95% confidence interval are indicated by the diamonds. Where two versions of the same study are mentioned, results from two different interventions were reported: Shahar 2013 (61): (1) no resistance exercise, (2) resistance exercise. Tieland (2012 A (65) & B (71)) are different studies published in the same year

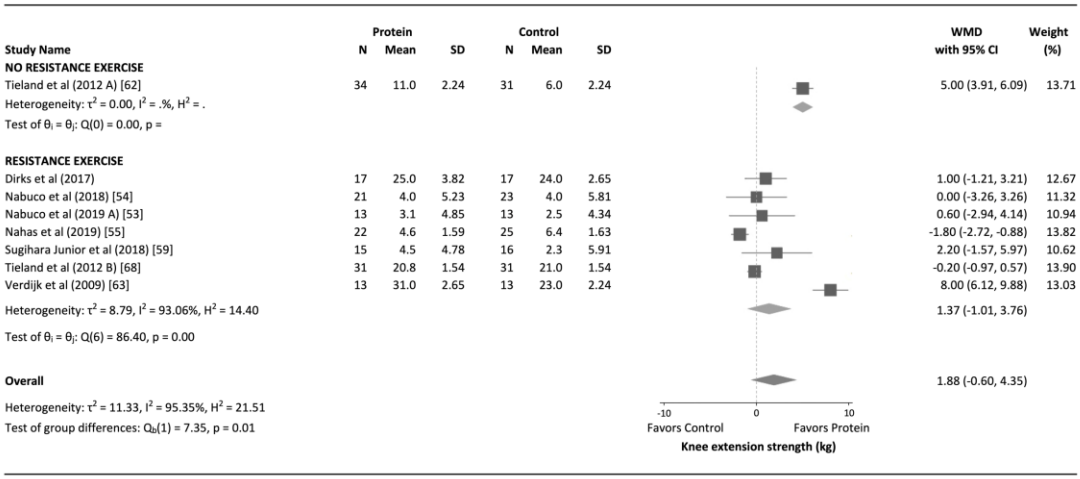

FIGURE 5 Forest plot of standard difference between control and protein groups on knee extension strength of 8 intervention arms organized by trials that did not, and did, use RE. A random effects model (using the DerSimonian-Laird method) and the generic inverse variance method were used to derive pooled estimates across studies. Squares indicate the point estimate for each trial, with the size of the square proportional to the contribution of the study to the overall estimate. The overall estimate and 95% confidence interval are indicated by the diamond. Nabuco (2019 A (56) & B (70)) and Tieland (2012 A (65) & B (71)) are different studies published in the same year

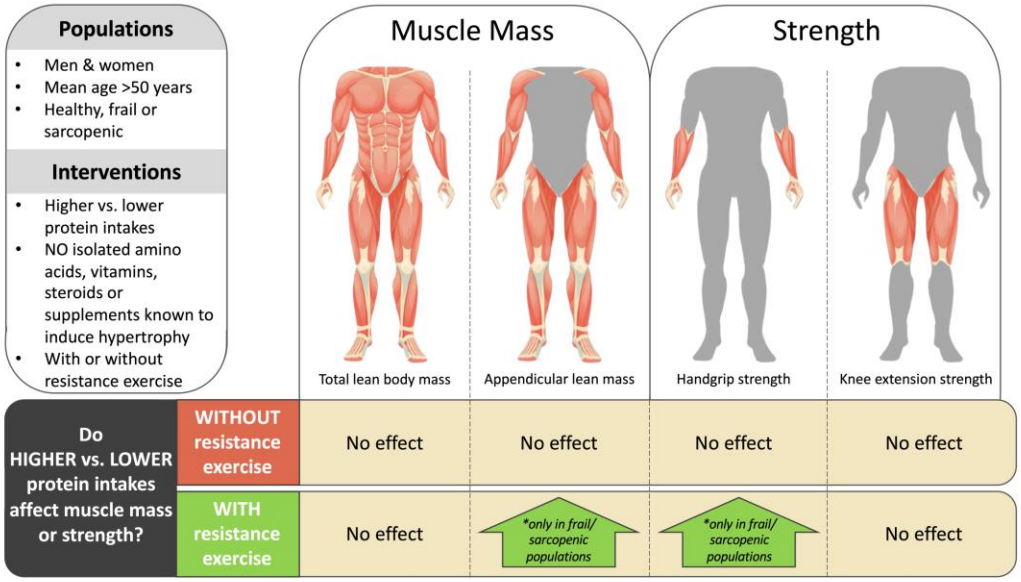

FIGURE 6 Summary diagram of the effects of higher vs. lower protein intakes on total lean body mass, appendicular lean mass, handgrip strength and knee extension strength in older adults