Protein supplementation augments the adaptive response of skeletal muscle to resistance-type exercise training: a meta-analysis[1–3]

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ABSTRACT

Background: Protein ingestion after a single bout of resistance-type exercise stimulates net muscle protein accretion during acute postexercise recovery. Consequently, it is generally accepted that protein supplementation is required to maximize the adaptive response of the skeletal muscle to prolonged resistance-type exercise training. However, there is much discrepancy in the literature regarding the proposed benefits of protein supplementation during prolonged resistance-type exercise training in younger and older populations.

Objective: The objective of the study was to define the efficacy of protein supplementation to augment the adaptive response of the skeletal muscle to prolonged resistance-type exercise training in younger and older populations.

Design: A systematic review of interventional evidence was performed through the use of a random-effects meta-analysis model. Data from the outcome variables fat-free mass (FFM), fat mass, type I and II muscle fiber cross-sectional area, and 1 repetition maximum (1-RM) leg press strength were collected from randomized controlled trials (RCTs) investigating the effect of dietary protein supplementation during prolonged (>6 wk) resistance-type exercise training.

Results: Data were included from 22 RCTs that included 680 subjects. Protein supplementation showed a positive effect for FFM (weighted mean difference: 0.69 kg; 95% CI: 0.47, 0.91 kg; $P < 0.00001$) and 1-RM leg press strength (weighted mean difference: 13.5 kg; 95% CI: 6.4, 20.7 kg; $P < 0.005$) compared with a placebo after prolonged resistance-type exercise training in younger and older subjects.

Conclusion: Protein supplementation increases muscle mass and strength gains during prolonged resistance-type exercise training in both younger and older subjects. *Am J Clin Nutr* 2012;96:1454–64.

INTRODUCTION

It has been well established that ingestion of dietary protein after resistance-type exercise increases postexercise muscle protein synthesis rates and inhibits muscle protein breakdown, thereby allowing net muscle protein accretion during the acute postexercise recovery period (1–3). As such, it is often suggested that dietary protein supplementation is required to maximize the adaptive response of skeletal muscle to more prolonged resistance-type exercise training. As a consequence, numerous recreational and competitive athletes habitually consume protein-containing supplements during and/or after exercise. However, there is conflicting evidence for the proposed surplus benefits of dietary protein supplementation on the increase in muscle mass and strength during prolonged resistance-type exercise training. Whereas some studies report greater gains in fat-free mass (FFM)[4], muscle fiber size, and/or muscle strength after protein supplementation during prolonged resistance-type exercise training (4–15), others (16–34) failed to confirm such benefits. The apparent discrepancy in the literature may be attributed to the numerous differences in study design variables, including, but not limited to, duration of the exercise intervention, training status, age of the population studied, and the amount, type, and timing of protein supplementation.

Besides the obvious perspective of the athlete aiming to improve muscle reconditioning, the proposed benefits of protein supplementation on the adaptive response of skeletal muscle to prolonged resistance-type exercise training has many clinical implications. Aging is associated with a progressive loss of skeletal muscle mass and strength, which leads to the loss of functional capacity and a greater risk of developing chronic metabolic disease (35–37). The age-related loss of muscle mass is a process caused by a combination of factors, which include a more sedentary lifestyle and an inadequate dietary protein intake (38–40). Resistance-type exercise training has been established as an effective interventional strategy to prevent or even reverse the age-related loss of skeletal muscle mass and strength in the aging population (41). Because the muscle protein synthetic response to nutritional (42, 43) and/or exercise (44, 45) stimuli has been reported to be blunted in senescent muscle, it is of even greater importance to define dietary interventions that can augment the benefits of exercise training in the elderly population. The few studies that have investigated the effect of protein supplementation during prolonged resistance-

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[4] Abbreviations used: CSA, cross-sectional area; FFM, fat-free mass; FM, fat mass; 1-RM, 1 repetition maximum.

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type exercise training in elderly cohorts have failed to confirm the proposed surplus benefits of protein supplementation on muscle mass and strength gains (26, 27, 30, 33, 34, 46–49). Whether this is simply attributed to the concept of anabolic resistance (42, 43) or due to the relatively small cohorts that were studied remains unclear.

Therefore, on the basis of the available literature, it is difficult to evaluate whether protein supplementation has any surplus benefits on the gain in muscle mass and/or strength during a prolonged resistance-type exercise training intervention. A meta-analysis was performed to provide evidence on the proposed effect of dietary protein supplementation as a means to augment the gains in FFM, fat mass (FM), muscle fiber type–specific cross-sectional area (CSA), and/or muscle strength using a 1-repetition maximum (1-RM) strength test during a prolonged resistance-type exercise training intervention in healthy younger adults, trained athletes, and older populations.

SUBJECTS AND METHODS

This meta-analysis was conducted in accordance with the recommendations and criteria as outlined by Moher and Tricco (50) for systematic reviews in the nutrition field, in line with the criteria outlined in the Preferred Reporting Items for Systematic Reviews and Meta-Analyses statement. The respective procedures that were incorporated during this meta-analysis, including the identification, screening, eligibility, and inclusion/exclusion of studies, were all agreed on between the authors in advance. No protocol for this review has been published.

Criteria for study consideration: types of studies and subjects

All randomized controlled trials that combined prolonged resistance-type exercise training with protein ingestion (through either supplementation or by increasing the protein content of the diet) with primary outcome variables related to FFM, FM, muscle fiber CSA, and/or 1-RM strength were included in the original article acquisition. Only healthy adult subjects ($>$18 y) with a BMI (in kg/m^2) $<$30 were included in the meta-analysis. We did not restrict our search for sex or training status, but recorded these variables as prespecified factors for subgroup analyses.

Criteria for study consideration: types of interventions and outcome measures

Studies including at least one subject group that was supplemented with protein, or consuming a higher protein diet ($>1.2 \text{ g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$) in combination with a prolonged resistance-type exercise training program, were considered for inclusion. We included studies that applied prolonged resistance-type exercise training for $\geq$6 wk with a minimum of 2 exercise sessions/wk (51). Study inclusion for the outcome improvements related to body composition were limited to 3 discrete measurements of FFM and FM, including hydrodensitometry (underwater weighing), whole-body air plethysmography (Bodpod), and dual energy X-ray absorptiometry. Study inclusion for muscle fiber hypertrophy was limited to measurements of muscle fiber type–specific CSA (type I and type II fibers) measured by histochemical analysis. Fiber type–specific analysis was selected in an effort to determine any potential fiber type–specific hypertrophy. Study inclusion for the outcome improvements related to 1-RM strength were limited to 3 discrete measurements of maximal strength capacity, including 1-RM strength tests for leg press, and/or leg extension and/or bench press. Other methods for assessing functional improvements in strength (eg, power and endurance performance) were not included in the analysis. All methods for measuring FFM, FM, CSA, and 1-RM strength were selected because of their documented validity and reliability as well as their reported prevalence in the literature (52–55).

Search strategy and study identification

A computerized search of the literature was performed in May 2011 by using the PubMed database (http://www.ncbi.nlm.nih.gov/pubmed/). In addition, we reviewed key exercise and physiology journals and reference lists of other relevant literature reviews for further pertinent studies. Only studies published in English-language journals were included. Abstracts from annual scientific conferences, commentaries, reviews, or duplicate publications from the same study were not included in this analysis. The preliminary search yielded 3122 relevant citations. After all 3122 abstracts were obtained and read by the 2 primary reviewers (NMC and PTR), 139 relevant articles met our study consideration criteria. The full text of all relevant articles was then obtained and examined by the 2 primary reviewers.

Study eligibility and data extraction

All included research articles contained a resistance-type exercise training intervention ($>$6 wk) with at least one subject group receiving a protein supplement or a modified higher protein diet. Additionally, each research study needed to include a placebo group that received a nonprotein supplement, lower protein diet, and/or exercise training without any nutritional co-intervention. A study (or group) was excluded if *1*) the intervention was designed to treat a given disorder or disease, *2*) the protein supplement was given in combination with other supplements known to augment muscle hypertrophy [eg, creatine (56)], and *3*) no relevant outcome variables were measured that used our predetermined measurement techniques. Data were extracted by using a predetermined data extraction file. Although all eligible studies in this meta-analysis shared a common directive, several studies examined slightly different hypotheses. Three studies compared the effect of supplementing different protein sources compared with a placebo on the adaptive response to prolonged exercise training (5, 7, 20), whereas one study compared the timing of protein supplementation on the adaptive response to prolonged exercise training (29). In these studies, the various groups receiving different protein supplements or protein at different time points were merged to form a single group and then compared with the placebo group. For each included study, the corresponding author was contacted if any missing data or information needed to be obtained (19 studies). If the corresponding authors could not be reached (5 studies) or if the data were no longer available, the study (or group, or outcome measurement) was excluded from the meta-analysis.

Assessment of reviewer agreement and risk of bias for included studies

Two reviewers (NMC and PTR) worked independently and screened all citations for eligibility. Potential abstracts were then retrieved in full text for evaluation against the predetermined inclusion/exclusion criteria. All studies were evaluated for study quality (blinding and subject dropouts, funding disclosures) independently by the 2 primary reviewers (NMC and PTR). Interreviewer disagreements were resolved by consensus. The agreement rate before amending any such discrepancies was assessed by using the κ statistic (57) and was determined to be 0.82. To assess for evidence of publication bias, Begg's funnel plots were visually inspected (58) for each outcome variable (FFM, FM, CSA, and 1-RM strength).

Tests for heterogeneity

Heterogeneity refers to the existence of variation between studies for each main effect being evaluated. Effect sizes are presented as weighted mean differences with 95% CIs. The chi-square method was used to assess heterogeneity. Because of the low power of a chi-square test when studies have a small sample size or are few in number, significance was set at $P < 0.10$. Heterogeneity was also assessed with I^2. This procedure quantifies the proportion of variability in the results that are due to a function of heterogeneity, rather than by chance. With this method, I^2 ranges from 0% to 100%, such that 0% reflects homogeneity and 100% indicates substantial heterogeneity. When I^2 values >75% were present, meta-analytic pooling was not performed (59).

Data syntheses

Treatment effects were calculated for each study after the extraction of mean differences [presupplementation (pre) and resistance-type exercise training subtracted from the postsupplementation (post) and resistance-type exercise training] and SDs of each group. Specifically, the SD of change was needed to calculate the pooled effect size. For those studies in which no raw data were available to calculate the SD of change, the following calculation was used:

$$\text{SD change} = \sqrt{\left[(\text{SD}_{pre})^2 + (\text{SD}_{post})^2 - 2 \times \text{corr}\,(\text{pre}, \text{post}) \times \text{SD}_{pre} \times \text{SD}_{post}\right]} \tag{1}$$

The correlation factor (corr) represents the mean of the available correlations from studies in which the SD change was accessible. This resulted in correlation coefficients of 0.98 (protein) and 0.98 (placebo) for FFM, 0.98 and 0.98 for FM, 0.74 and 0.86 for type I CSA, 0.85 and 0.83 for type II CSA, and 0.70 and 0.80 for 1-RM strength in the protein and placebo groups, respectively.

Effect sizes

The analyses of pooled data were conducted with a random-effects model to account for measurement variability among the included studies. For each outcome, a forest plot was generated to illustrate the study-specific effect size and their respective 95% CIs. In each study, the effect size for the intervention was calculated by the difference between the means of the posttest and pretest at the end of the resistance-type training program. All calculations were performed with RevMan (Review Manager-Version 5.1; The Cochrane Collaboration, 2011).

Sensitivity analysis

Two sensitivity analyses were performed to determine whether the FFM findings were dependent on *1*) the selected age-range cutoffs for younger and older subjects and *2*) the different types and sources of supplemented protein.

RESULTS

Study characteristics

A total of 22 studies, reporting results from 46 groups, met all the inclusion criteria and were included in the review (**Figure 1**). The publication dates ranged from 1995 to 2010.

Subject characteristics

Data from 680 subjects with an age range between 19 and 72 y [mean (±SD): 33 ± 18 y] were included in the analysis (**Table 1**). Six studies (12 groups) were conducted in older (>50 y) subjects, and 16 studies (34 groups) were conducted in younger (<50 y) subjects. Of 16 studies in the young, 5 studies (10 groups) were conducted in subjects with a history of resistance-type exercise training, and 11 studies (24 groups) included previously untrained subjects. Of the included studies, 4 studies (8 groups) were conducted in women only, 3 studies (6 groups) were conducted in a mixed population, and 15 studies (32 groups) were conducted in men only.

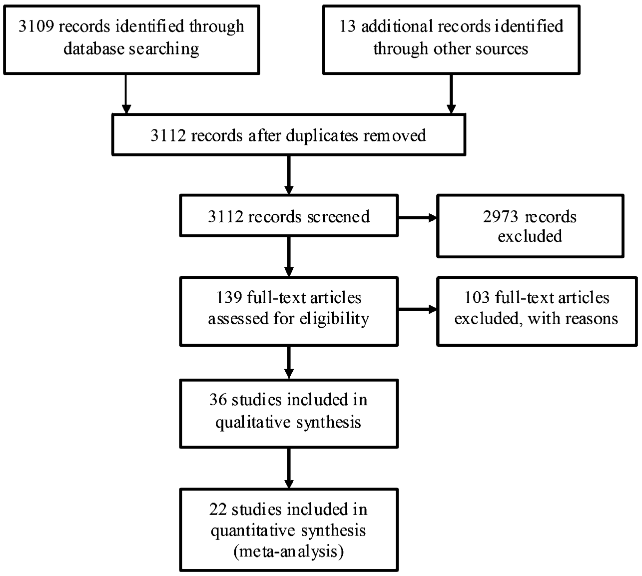

FIGURE 1. Flow of papers through the review process.

TABLE 1
Details of the included studies[1]

			Training details			Protein and placebo supplementation details			
Author, year	Age	Fitness	Study length	Type RT	RT intensity	Type of protein	Amount	Placebo type	Isocaloric placebo
							g/d		
Antonio et al, 2000 (24)	Young	Untrained	3 d/wk × 6 wk	WBR + aerobic	6 to12-RM	EAA	26	CHO	N
Ballard et al, 2006 (25)	Young	Untrained	5 d/wk × 24 wk	WBR + aerobic	70% 1-RM	Whey + casein	84	CHO	Y
Bemben et al, 2010 (26)	Older	Untrained	3 d/wk × 14 wk	WBR	80% 1-RM	Whey	35	CHO	N
Bird et al, 2006a (4)	Young	Untrained	2 d/wk × 12 wk	WBR	75% 1-RM	EAA	6	Water	N
Bird et al, 2006b (4)	Young	Untrained	2 d/wk × 12 wk	WBR	75% 1-RM	EAA	6	CHO	N
Campbell et al, 1995 (27)	Older	Untrained	3 d/wk × 12 wk	WBR	80% 1-RM	Milk (diet)	63	Low-protein diet	Y
Cribb et al, 2007 (13)	Young	Trained	3 d/wk × 11 wk	WBR	High	Whey	90	CHO	Y
Hartman et al, 2007 (5)	Young	Untrained	5 d/wk × 12 wk	WBR	80% 1-RM	Milk + soy	35	CHO	Y
Hoffman et al, 2007 (28)	Young	Trained	4 d/wk × 12 wk	WBR	6 to 10-RM	Milk + whey concentrate + egg white	84	CHO	Y
Hoffman et al, 2009 (29)	Young	Trained	4 d/wk × 10 wk	WBR	6 to 10-RM	Whey + casein	84	Exercise only	N
Holm et al, 2008 (30)	Older	Untrained	2–3 d/wk × 24 wk	Legs only	8 to 20-RM	Whey	10	CHO	N
Hulmi et al, 2009 (31)	Young	Untrained	2 d/wk × 21 wk	WBR	40–85% 1-RM	Whey	30	Water	N
Iglay et al, 2009 (32)	Older	Untrained	3 d/wk × 12 wk	WBR	80% 1-RM	Egg + meat + dairy (diet)	20	Low-protien diet	Y
Josse et al, 2010 (6)	Young	Untrained	5 d/wk × 12 wk	WBR	80–90% 1-RM	Milk	36	CHO	Y
Kerksick et al, 2006 (7)	Young	Trained	4 d/wk × 10 wk	WBR	6 to 10-RM	Whey + casein	48	CHO	Y
Kukuljan et al, 2009 (33)	Older	Untrained	3 d/wk × 17 wk	WBR	50–80% 1-RM	Milk	13	Exercise only	N
Mielke et al, 2009 (17)	Young	Untrained	3 d/wk × 8 wk	Arms + legs	80% 1-RM	Whey + leucine	52	CHO	Y
Rozenek et al, 2002 (19)	Young	Untrained	4 d/wk × 8 wk	WBR	70% 1-RM	Whey + casein	106	CHO	Y
Verdijk et al, 2009 (34)	Older	Untrained	3 d/wk × 12 wk	Legs only	60–80% 1-RM	Casein	20	Water	N
Walberg et al, 2004 (18)	Young	Untrained	3 d/wk × 10 wk	WBR	55–97% 1-RM	Chocolate milk	16	CHO	Y
Walker et al, 2010 (8)	Young	Trained	3 d/wk × 8 wk	WBR + aerobic	High	Whey + leucine	52	CHO	Y
White et al, 2009 (20)	Young	Untrained	3 d/wk × 8 wk	WBR	5 to 7-RM	Whey + casein	15	CHO	N
Willoughby et al, 2007 (9)	Young	Untrained	4 d/wk × 10 wk	WBR	85–90% 1-RM	Whey + casein	40	CHO	Y

[1] Individual study details regarding subject characteristics (age and fitness level), resistance-type exercise training characteristics (length, type, and intensity), and protein and placebo supplementation details (type and amount of protein supplemented on training days, type of placebo, and whether the placebo was isocaloric) for studies included in the meta-analysis. CHO, carbohydrate; EAA, essential amino acid; N, no; RM, repetition maximum; RT, resistance training; WBR, whole-body resistance training; Y, yes.

Resistance-type exercise training characteristics

The total duration of the resistance-type exercise training program varied from 6 to 24 wk, with a mean (±SD) of 12 ± 5 wk. The number of exercise training sessions per week ranged from 2 to 5, with a mean of 3 ± 1 per week. With specific reference to the type of resistance-type exercise training performed, 18 studies (38 groups) performed whole-body exercise training, 2 studies (4 groups) performed leg exercise only, 1 study (2 groups) performed only 2 exercises (leg and bench press), and 1 study (2 groups) used a combination of resistance and endurance-type exercise training (Table 1).

Protein supplementation characteristics

The mean (±SD) amount of protein provided either by supplement or via the habitual diet on training days was 42 ± 30 g (range: 6–106 g). Twelve studies supplemented with a combination of whey, casein, and/or milk proteins; 6 studies supplemented with whey protein; 2 studies supplemented with essential amino acids; 1 study supplemented with casein protein only; and 1 study manipulated the diet with egg protein. On the training days, 15 studies supplemented the protein immediately before, during, and/or after the exercise session. In the placebo groups, 13 studies used an isocaloric placebo, whereas 7 studies used a nonisocaloric placebo and 2 studies used exercise only (Table 1).

Publication bias and heterogeneity

Considerable symmetry was observed on examining Begg's funnel plots for each of the 4 outcome measures, which implied that there was no publication bias. For the 4 outcome measures presented, χ^2 and I^2 were 18.3 and 0% for FFM, 39.6 and 52% for FM, 10.5 and 24% for type I CSA, 18.4 and 57% for type II CSA, and 13.8 and 13% for 1-RM leg press strength, respectively, which indicated little to moderate heterogeneity. As previously mentioned, leg extension and bench press strength data were also extracted, but the heterogeneity was too high ($I^2 = 91\%$ and 92%, respectively) to report any pooled estimate.

Intervention effect

An overview of the characteristics for all studies included in the meta-analysis is provided in Table 1, and significant effects of the individual studies are presented in **Table 2**. Each outcome measure (FFM, FM, CSA, and 1-RM strength) was independently assessed through the meta-analytic procedure and is presented sequentially. Many studies reported more than a single outcome, but only outcomes relevant to this meta-analysis are reported. With respect to the individual studies included in the meta-analysis, the ranges of effect sizes in the younger subjects were −0.1 to 1.5 for FFM, −2.4 to 1.0 for FM, 0.2 to 1.3 for type I CSA, 0.3 to 1.2 for type II CSA, and 0.05 to 1.5 for 1-RM leg press strength. The ranges of effect sizes in the older subjects were 0.1 to 0.6 for FFM, −0.18 to 0.24 for FM, −0.8 to 0.6 for

TABLE 2
Individual study results included in the meta-analysis[1]

			Outcome measures													
Author, year	Age	Fitness	FFM	Protein	Placebo	FM	Protein	Placebo	1-RM	Protein	Placebo	CSA type I	CSA type II	Protein	Placebo
				n	*n*		*n*	*n*		*n*	*n*			*n*	*n*
Antonio et al, 2000 (24)	Young	Untrained	→	10	9	→	10	9							
Ballard et al, 2006 (25)	Young	Untrained	→	29	21	→	29	21							
Bemben et al, 2010 (26)	Older	Untrained	→	11	10	→	11	10	→	11	10				
Bird et al, 2006a (4)	Young	Untrained	→	8	8	→	8	8	→	8	8	→	↑	8	8
Bird et al, 2006b (4)	Young	Untrained	→	8	8	→	8	8	→	8	8	→	↑ (IIa)	8	8
Campbell et al, 1995 (27)	Older	Untrained	→	6	6	→	6	6				→	→	6	6
Cribb et al, 2007 (13)	Young	Trained	→	5	7	→	5	7				→	→	5	7
Hartman et al, 2007 (5)	Young	Untrained	↑	37	19	↓	37	19	→	37	19	↑	↑	37	19
Hoffman et al, 2007 (28)	Young	Trained	→	11	10	→	11	10							
Hoffman et al, 2009 (29)	Young	Trained	→	26	7	→	26	7							
Holm et al, 2008 (30)	Older	Untrained	→	13	16	→	13	16				→	→	13	16
Hulmi et al, 2009 (31)	Young	Untrained							→	11	10	→	→	9	9
Iglay et al, 2009 (32)	Older	Untrained	→	18	18	→	18	18	→	18	16	→	→	16	15
Josse et al, 2010 (6)	Young	Untrained	↑	10	10	↓	10	10	↑	10	10				
Kerksick et al, 2006 (7)	Young	Trained	↑	25	11	→	25	11	→	25	11				
Kukuljan et al, 2009 (33)	Older	Untrained	→	45	46	→	45	46							
Mielke et al, 2009 (17)	Young	Untrained	→	13	13	→	13	13							
Rozenek et al, 2002 (19)	Young	Untrained	→	26	25	→	26	25	→	26	25				
Verdijk et al, 2009 (34)	Older	Untrained	→	13	13	→	13	13	→	13	13	→	→	13	12
Walberg et al, 2004 (18)	Young	Untrained	→	10	9	→	10	9	→	10	9				
Walker et al, 2010 (8)	Young	Trained	↑	18	12	→	18	12							
White et al, 2009 (20)	Young	Untrained	→	10	10	→	10	10	→	14	14				
Willoughby et al, 2007 (9)	Young	Untrained	↑	10	9	→	10	9	↑	10	9				

[1] CSA, type I and II cross-sectional area; FFM, fat-free mass; FM, fat mass; 1-RM = 1-repetition maximum strength; →, nonsignificant differences in the protein compared with the placebo treatment; ↑, significant increase in the protein compared with the placebo treatment; ↓, significant decrease in the protein compared with the placebo treatment.

type I CSA, −0.4 to 0.03 for type II CSA, and 0.1 to 0.9 for 1-RM leg press strength.

FFM and FM

Compared with the placebo, protein supplementation significantly augmented the gain in FFM during prolonged resistance-type exercise training (weighted mean difference: 0.69 kg; 95% CI: 0.47, 0.91 kg; $P < 0.00001$; **Figure 2**). Subgroup analysis for age showed that protein supplementation had a similar effect on improving FFM between younger (pooled estimate = 0.81 kg; 95% CI: 0.53, 1.1 kg; $P < 0.00001$) and older (pooled estimate = 0.48 kg; 95% CI: 0.10, 0.85 kg; $P < 0.01$; Figure 2) subjects. In the younger subjects, further subgroup analysis for training status showed a similar effect of protein supplementation on improving FFM between younger untrained (pooled estimate = 0.75 kg; 95% CI: 0.42, 1.1 kg; $P < 0.00001$) and trained (pooled estimate = 0.98 kg; 95% CI: 0.45, 1.5 kg; $P < 0.001$; **Figure 3**) subjects. Compared with the placebo, protein supplementation did not significantly augment FM loss during prolonged resistance-type exercise training in the younger or older subjects (weighted mean difference: −0.11 kg; 95% CI: −0.50, 0.29; $P > 0.05$; **Figure 4**).

Type I and II muscle fiber CSA

Compared with the placebo, protein supplementation significantly augmented the gain in mean type I muscle fiber CSA during prolonged resistance-type exercise training (weighted mean difference: 212 μm^2; 95% CI: 109, 315 μm^2; $P < 0.0001$; **Figure 5**A). However, subgroup analysis for age showed that, when compared with the placebo intervention, protein supplementation significantly augmented the gain in mean type I muscle fiber CSA during prolonged resistance-type exercise training in younger subjects only (pooled estimate = 241 μm^2; 95% CI: 131, 350 μm^2; $P < 0.0001$; Figure 5A). In older subjects, in comparison with the placebo, protein supplementation did not significantly improve the gain in mean type I muscle fiber CSA (pooled estimate = −17 μm^2; 95% CI: −324, 291 μm^2; $P = 0.92$; Figure 5A).

For type II muscle fibers, in comparison with the placebo group, protein supplementation significantly augmented the gain in mean type II muscle fiber CSA during prolonged resistance-type exercise training (weighted mean difference: 291 μm^2; 95% CI: 71.7, 510 μm^2; $P < 0.01$; Figure 5B). However, subgroup analysis for age showed that, in comparison with the placebo intervention, protein supplementation significantly augmented the gain in mean type II muscle fiber CSA during prolonged resistance-type exercise training in younger subjects only (pooled estimate = 477 μm^2; 95% CI: 333, 620 μm^2; $P < 0.00001$; Figure 5B). In older subjects, protein supplementation did not show a greater improvement in type II muscle fiber CSA compared with the placebo (pooled estimate = −132 μm^2; 95% CI: −410, 147 μm^2; $P = 0.35$; Figure 5B). An insufficient number of studies are available to perform a subgroup analysis on trained compared with untrained younger subjects for type I or II muscle fiber CSA.

1-RM strength

Protein supplementation significantly improved the gain in mean 1-RM leg press strength during prolonged resistance-type exercise training (weighted mean difference: 13.5 kg; 95% CI: 6.4, 20.7 kg; $P < 0.001$; **Figure 6**) when compared with the placebo intervention. Subgroup analysis for age showed that protein supplementation had a similar effect on improving 1-RM leg press strength in both the younger (pooled estimate = 14.4 kg; 95% CI: 5.2, 23.6 kg; $P < 0.01$) and older (pooled estimate = 13.1 kg; 95% CI: 0.32, 25.9 kg; $P < 0.05$; Figure 6) subjects. An insufficient number of studies are available to perform a subgroup analysis on trained compared with untrained younger subjects.

Sensitivity analysis

Two sensitivity analyses were performed to determine whether the FFM findings were dependent on *1*) the selected age-range cutoffs for younger and older subjects and *2*) the different types and sources of supplemented protein. To perform the first sensitivity analysis, we increased the lower age limit for older adults to

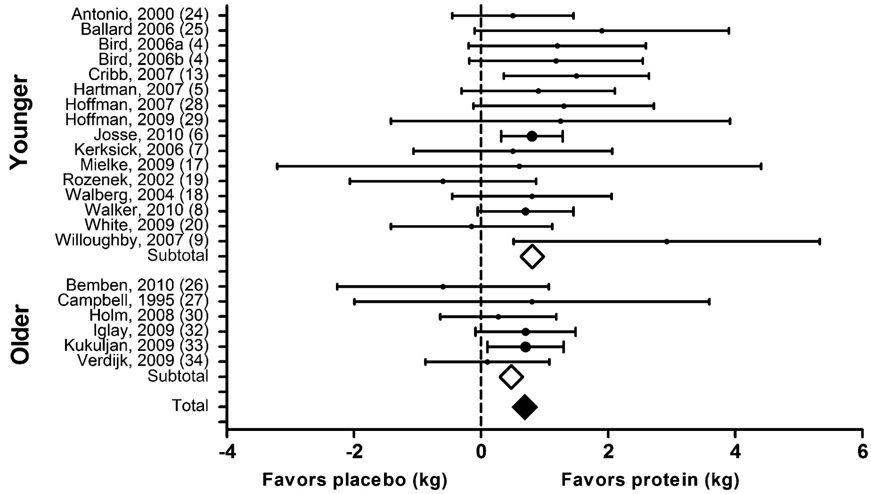

FIGURE 2. Forest plot of the results of a random-effects meta-analysis shown as pooled mean differences with 95% CIs on fat-free mass in younger and older subjects (weighted mean difference: 0.69 kg; 95% CI: 0.47, 0.91 kg; $P < 0.00001$). For each study, the shaded circle represents the point estimate of the intervention effect. The horizontal line joins the lower and upper limits of the 95% CI of this effect. The area of the shaded circles reflects the relative weight of the study in the meta-analysis. The diamonds represent the subgroup mean difference (◇) and pooled mean difference (◆).

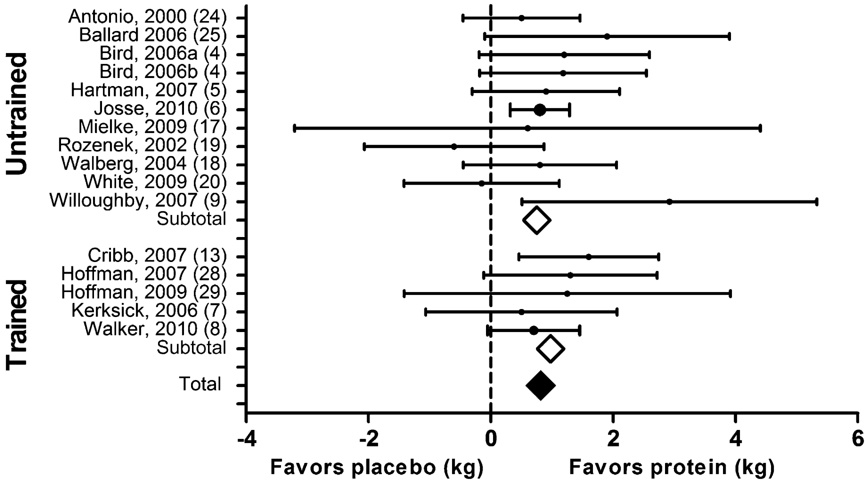

FIGURE 3. Forest plot of the results of a random-effects meta-analysis shown as pooled mean differences with 95% CIs on fat-free mass in younger untrained and younger trained subjects (weighted mean difference: 0.81 kg; 95% CI: 0.53, 1.1 kg; $P < 0.00001$). For each study, the shaded circle represents the point estimate of the intervention effect. The horizontal line joins the lower and upper limits of the 95% CI of this effect. The area of the shaded circles reflects the relative weight of the study in the meta-analysis. The diamonds represent the subgroup mean difference (◇) and pooled mean difference (◆).

60 y (instead of 50 y). To perform the second sensitivity analysis, we included only those studies that supplemented with combinations of milk-based proteins or those studies that supplemented with single protein sources. In both sensitivity analyses, the findings were similar to those of the primary meta-analysis, which suggests that the overall FFM outcome was not dependent on the selected age-range cutoffs or the different types and sources of supplemented protein.

DISCUSSION

This was the first meta-analytic review to examine the effect of dietary protein supplementation on the adaptive response of skeletal muscle to prolonged resistance-type exercise training in healthy younger and older subjects. Pooled estimates showed that protein supplementation during prolonged (>6 wk) resistance-type exercise training significantly augments the gains in FFM, type I and II muscle fiber CSA, and 1-RM leg press strength compared with resistance-type exercise training without a dietary protein based cointervention.

It has been well established that amino acid and/or protein administration after resistance-type exercise stimulates muscle protein synthesis rates, which results in net muscle protein accretion (3, 60–65). These findings support the general opinion that protein supplementation can augment the adaptive response of skeletal muscle to prolonged resistance-type exercise training, which results in greater gains in muscle mass and/or strength. However, there is much discrepancy in the literature that is likely attributed to the differences in study design, selected population, timing, and type and amount of supplemented protein. This meta-analysis tries to resolve the conflicting evidence by assessing the effect of dietary protein supplementation on

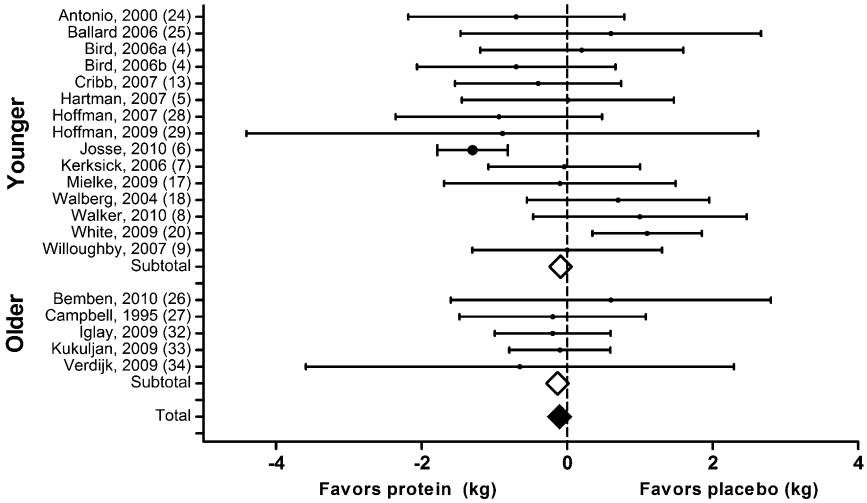

FIGURE 4. Forest plot of the results of a random-effects meta-analysis shown as pooled mean differences with 95% CIs on fat mass in younger and older subjects (weighted mean difference: −0.11 kg; 95% CI: −0.50, 0.29 kg; $P > 0.05$). For each study, the shaded circle represents the point estimate of the intervention effect. The horizontal line joins the lower and upper limits of the 95% CI of this effect. The area of the shaded circles reflects the relative weight of the study in the meta-analysis. The diamonds represent the subgroup mean difference (◇) and pooled mean difference (◆).

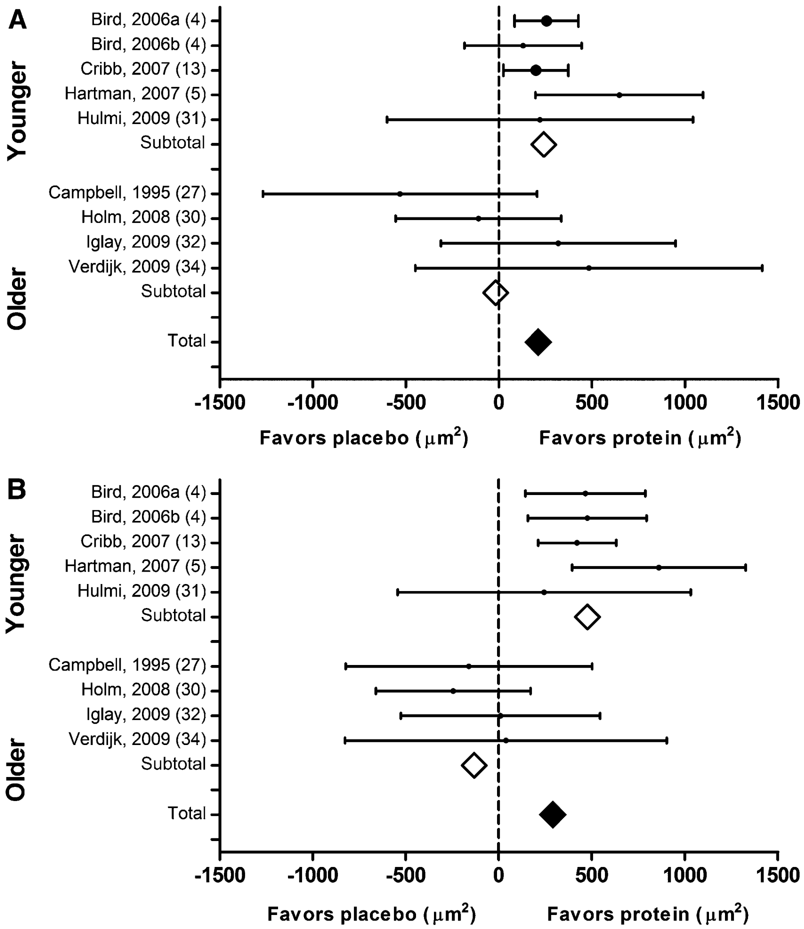

FIGURE 5. Forest plot of the results of a random-effects meta-analysis shown as pooled mean differences with 95% CIs on type I cross-sectional area (A) (weighted mean difference: 212 μm^2; 95% CI: 109, 315 μm^2; $P < 0.0001$) and type II cross-sectional area (B) (weighted mean difference: 291 μm^2; 95% CI: 71.7, 510 μm^2; $P < 0.01$) in younger and older subjects. For each study, the shaded circle represents the point estimate of the intervention effect. The horizontal line joins the lower and upper limits of the 95% CI of this effect. The area of the shaded circles reflects the relative weight of the study in the meta-analysis. The diamonds represent the subgroup mean difference (◇) and pooled mean difference (◆).

gains in skeletal muscle mass and strength after prolonged resistance-type exercise training in 680 subjects.

During the meta-analytic procedure, we first assessed changes in FFM in younger adults. Protein supplementation resulted in ~1-kg greater gains in FFM after 12 ± 1 wk of resistance-type exercise training when compared with training without additional protein supplementation. The latter findings were evident despite the fact that, before the intervention, all groups were already consuming a more than adequate dietary protein intake of ~1.2 g · kg^{-1} · d^{-1} (66, 67). Subjects were supplemented with an average of 50 ± 32 g protein/d (on top of their normal diet), and, in most cases, the protein supplements were ingested before or immediately after each exercise session. When the younger subject groups were stratified for training status, resistance-trained groups were shown to be even more responsive to protein supplementation with respect to changes in their FFM as compared with their untrained counterparts. Resistance-trained subjects supplementing with protein showed a >4 fold gain in FFM when compared with the placebo group. These results suggest that, in resistance-trained subjects, protein supplementation is required to maximize the anabolic response to prolonged resistance-type exercise training. Besides FFM, we also included studies that measured muscle fiber type–specific CSA and 1-RM leg press strength to determine whether the adaptive response of skeletal muscle to exercise training and protein supplementation could be modulated at the muscle fiber level. Unfortunately, there is limited data available with respect to muscle fiber type–specific CSA, which is likely because of the more invasive nature of the muscle biopsy collection procedure and the required expertise to allow for proper histochemical analysis. In the younger subjects, protein supplementation further increased type I and type II muscle fiber CSA by 45% and 54%, respectively, after prolonged resistance-type exercise training when compared with the placebo group. The greater increase in muscle fiber CSA with dietary protein supplementation was accompanied by a 20% greater increase in 1-RM leg press strength.

Similar to the younger groups, FFM was the most widely reported outcome in the older groups with data from 215 subjects. When studies were examined individually, not a single study reported a significant benefit of protein supplementation on the gain in FFM when compared with a placebo. Once the data were pooled, however, it became evident that dietary protein supplementation during resistance-type exercise training increased FFM by an additional 38% when compared with the placebo.

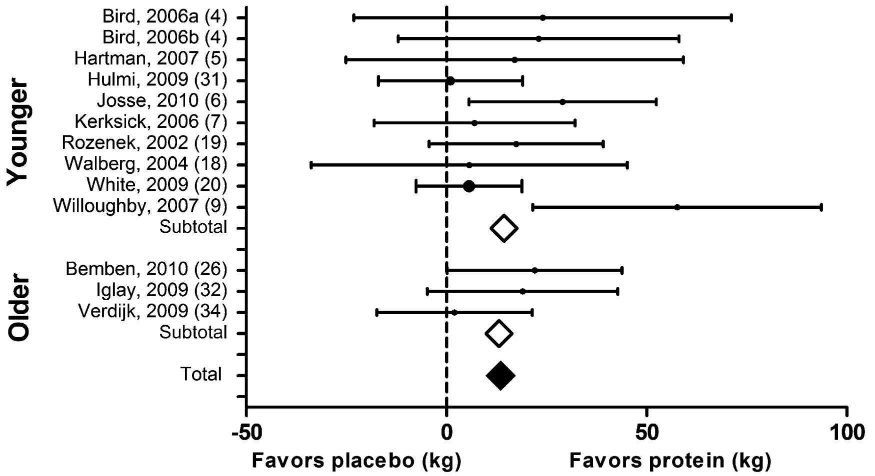

FIGURE 6. Forest plot of the results of a random-effects meta-analysis shown as pooled mean differences with 95% CI on 1-repetition maximum leg press in both younger and older subjects (weighted mean difference: 13.5 kg; 95% CI: 6.4, 20.7 kg; $P < 0.001$). For each study, the shaded circle represents the point estimate of the intervention effect. The horizontal line joins the lower and upper limits of the 95% CI of this effect. The area of the shaded circles reflects the relative weight of the study in the meta-analysis. The diamonds represent the subgroup mean difference (◇) and pooled mean difference (◆).

Interestingly, additional protein supplementation did not seem to have a significantly greater effect on the exercise-induced increase in type I (22%) and II (−19%) muscle fiber CSA in the older group, despite the fact that the older protein-supplemented group showed a 33% greater increase in 1-RM leg press strength. However, note that only 4 included studies measured muscle fiber CSA in tissue obtained from older adults (27, 30, 32, 34), thus providing a limited view of the effect of protein supplementation on muscle fiber CSA after prolonged resistance-type exercise training. Regardless, these results bear significant clinical relevance given the rapid loss of skeletal muscle mass and strength among sedentary individuals after the age of 50 y (68). Protein supplementation during an exercise intervention program can further increase the gains in muscle mass (+38%) and strength (+33%) within merely 3 mo of resistance-type exercise training. The greater increase in muscle mass and strength will allow the older individuals to more rapidly regain their functional capacity, thereby reducing the risk of falls and fractures and, as such, supporting a more active independent lifestyle.

It is important to note, however, that this meta-analysis included only 2 age categories. Groups were labeled as "younger" if they were 49 y or younger (mean age: 23 ± 3 y) and "older" if they were 50 y or older (mean age: 62 ± 6 y). Moreover, in an effort to reduce the heterogeneity between studies, only healthy subject groups were included in this meta-analysis. We speculate that subjects at a more advanced age (>65 y), and more specifically frail elderly, may demonstrate an even greater effect of protein supplementation on FFM during a period of resistance-type exercise training. These more frail elderly subpopulations generally consume insufficient amounts of dietary protein (38, 40). It is likely that the adaptive response of skeletal muscle to prolonged resistance-type exercise training is (more) restricted by the limited availability of dietary protein–derived amino acids as precursors for de novo muscle protein synthesis in the more clinically compromised elderly subpopulations.

Although the current study provides insight into the general outcome of the literature on the proposed effect of protein supplementation as a means to augment the benefits of prolonged resistance-type exercise training, meta-analytic data do not necessarily infer a causal effect. In an attempt to make the study treatment groups as homogenous as possible, various studies were omitted. Other limitations included the process of search and retrieval for eligible articles and the potential influence of publication bias (69). Despite these limitations, this meta-analysis provides a general overview on the research thus far and offers insight into the literature investigating the proposed benefits of protein supplementation to augment muscle mass and strength during prolonged resistance-type exercise training in younger and older adults. For future investigations, researchers may wish to examine specific variables with respect to the effect of dietary protein supplementation on the adaptive response of skeletal muscle to prolonged resistance-type exercise training. The latter may include the effect of the intensity of the exercise sessions, the type or source of dietary protein supplementation, and/or the timing of protein supplementation.

In conclusion, dietary protein supplementation represents an effective dietary strategy to augment the adaptive response of skeletal muscle to prolonged resistance-type exercise training in healthy younger and older adults. Dietary protein supplementation in younger adults further augments the gains in FFM, muscle fiber-type specific CSA, and 1-RM leg press strength after ∼3 mo of prolonged resistance-type exercise training. Because the gains in FFM and 1-RM leg press strength are also observed in an older population, it seems evident that protein supplementation represents an effective and robust strategy to improve the benefits of resistance-type exercise training to support healthy aging.

The authors' responsibilities were as follows—NMC, LCPGMdG, WHMS, and LJCvL: provided study oversight and wrote and took primary responsibility for the final content of the manuscript; and NMC and PTR: performed the data collection and statistical analyses. All authors designed the research, assisted in the interpretation of analyses and revision of the manuscript, and read and approved the final manuscript. None of the authors had a conflict of interest.

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