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Human Kinetics
SCHOLARLY REVIEW

Effects of Timing and Types of Protein Supplementation on Improving Muscle Mass, Strength, and Physical Performance in Adults Undergoing Resistance Training: A Network Meta-Analysis

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Precise protein supplementation strategies for muscle improvement are still lacking. The timing or type of protein supplementation has been debated as a window of opportunity to improve muscle mass, strength, and physical performance. We conducted a network meta-analysis of randomized controlled trials with protein supplements and resistance training. PubMed, Web of Science, Cochrane Library, and SPORTDiscus databases were searched until May 1, 2023. We included 116 eligible trials with 4,711 participants that reported on 11 timing and 14 types of protein supplementation. Compared with placebo, protein supplementation after exercise (mean difference [MD]: 0.54 kg [95% confidence intervals 0.10, 0.99] for fat-free mass, MD: 0.34 kg [95% confidence intervals 0.10, 0.58] for skeletal muscle mass) and at night (MD: 2.85 kg [0.49, 5.22] for handgrip strength, MD: 12.12 kg [3.26, 20.99] for leg press strength) was most effective in improving muscle mass and strength, respectively (moderate certainty). Milk proteins (milk, whey protein, yogurt, casein, and bovine colostrum), red meat, and mixed protein were effective for gains in both muscle mass and strength (moderate certainty). No timing or type of protein showed a significant enhancement in physical performance (timed up-to-go test, 6-min walk test, and gait speed). Pre/postexercise and Night are key recommended times of protein intake to increase muscle mass and strength, respectively. Milk proteins are the preferred types of protein supplements for improving muscle mass and strength. Future randomized controlled trials that directly compare the effects of protein timing or types are needed. This trial was registered at International Prospective Register of Systematic Reviews as CRD42022358766.

***Keywords**:* protein supplement, anaerobic exercise, muscular

As recommended by the International Society of Sports Nutrition, additional protein supplementation is needed to achieve a positive net protein balance during resistance training (RT; Jäger et al., 2017). The roles of protein supplementation in enhancing muscle mass, strength, and physical performance in adults undergoing RT have been well explored (Finger et al., 2015; Hou et al., 2019; Kirwan et al., 2022; Liao et al., 2017; Morton et al., 2018; Nunes et al., 2022). Nonetheless, some important issues in the precise application of protein supplementation to maximize beneficial effects on muscle for subjects undergoing RT, such as protein timing and recommended types, have not been well addressed. A popular strategy is that protein should be ingested before and/or after a training session and before sleep to maximally augment muscle protein synthesis (MPS) in a limited anabolic window (Jäger et al., 2017; Volek, 2004). In addition, types of protein may differently affect MPS rates, which tend to depend on leucine content, protein digestion, and absorption, as well as amino acid composition (Gwin et al., 2020).

Evidence from meta-analysis is limited and inconclusive in regard to the effects of protein timing on muscle. Wirth et al. conducted a subgroup analysis of 26 randomized controlled trials (RCTs). The results showed that there was no significant difference among protein supplementation after exercise (AE), before exercise (BE) and after exercise (AE), and not around exercise on muscle mass and strength (Wirth et al., 2020). A meta-regression with 23 RCTs found no significant effect of pre- and/or post-RT protein on muscle mass or strength compared with the control group in the fully adjusted model, although a small to moderate effect on muscle hypertrophy was found in the unadjusted model (Schoenfeld et al., 2013). Besides, Tang et al. (2009) found that MPS after consumption of whey protein (high leucine/fast digesting) was approximately 93% and 18% greater than casein (high leucine/slow digesting) and soy (lower leucine/intermediate digesting), respectively. It was also demonstrated that whey protein supplementation increased muscle strength by about 10% compared to casein during 135 days of RT program, while no difference in muscle mass between both groups was found (Karelis et al., 2015).

Since the number of studies that investigated the timing or type issues head-to-head is rather scant, it is difficult for traditional meta-analysis which relied on pairwise comparisons to draw conclusions. Network meta-analysis (NMA) is a useful tool to compare multiple treatments combining both direct and indirect evidence and to rank numerous interventions (Tonin et al., 2017). The purpose of this review was to conduct an NMA to determine which timing or type of protein supplementation is a viable strategy for enhancing muscle mass, strength, or physical performance in adults undergoing RT.

Methods

This meta-analysis was reported based on the NMA extension of the Preferred Reporting Items for Systematic Reviews and

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Meta-Analyses reporting guidelines (Hutton et al., 2015). The protocol of this study was registered in the International Prospective Register of Systematic Reviews (CRD42022358766).

Search Strategy

We searched PubMed, Web of Science, Cochrane Library, and SPORTDiscus from database inception until May 1, 2023. Search terms included RCTs, protein supplements, muscle mass, muscle strength, and physical performance among others. The detailed search strategy was presented in Table S1 (Supplementary Material [available online]). We also conducted a manual search for reference lists of relevant reviews and eligible publications.

Study Selection

Four authors (H.H. Zhou, Liao, X. Zhou, and Peng) independently screened titles and abstracts in the initial search, then the full text of all possible relevant articles was reviewed for eligibility. Disagreements were resolved by discussion with the senior investigator (Yang). The minimal duration of protein supplementation and RT was 2 weeks based on the previous study (Wirth et al., 2020). Inclusion criteria for this review were: (a) the study design was RCT; (b) the eligible population were adults who underwent RT during the trial; (c) interventions of interest were isolated protein or protein blends with timing or type was mentioned (only eligible when comparisons did not differ in RT intervention); (d) comparators included placebo, control, or other timing or types of protein supplementation which were different from the intervention group; and (e) outcomes of interest included muscle mass, muscle strength, and physical performance. Meantime, the exclusion criteria included: (a) participants with serious diseases that restrict physical activity; (b) reviews, case–control studies, cohort studies, nonhuman studies, non-English studies, and letters without sufficient data; (c) either or both of the comparator or the intervention group did not receive RT; (d) the duration of protein supplementation or RT <2 weeks; and (e) protein supplement was co-ingested with other potentially hypertrophic agents (e.g., creatine and calcium beta-hydroxy-beta-methylbutyrate).

Data Extraction

Two trained researchers (H.H. Zhou and Liao) independently extracted predetermined data elements. The data from each eligible study were extracted into a structured form, including the first author's surname, publication year, study design, sample size, mean age of participants, intervention duration, sex (percentage of women), body mass index of participants, RT information, protein supplementation information, and reported outcomes.

Data Items

Our analysis classified the timing of protein supplementation as BE (defined as within 2 hr BE), AE (defined as within 2 hr AE), Day (daytime, except for BE and AE), and Night (nighttime, except for BE and AE). The relevant analyses were a 13-node NMA for protein timing (BE vs. AE vs. Day vs. Night vs. AE + Day vs. AE + Night vs. BE + AE vs. Day + Night vs. BE + AE + Day vs. BE + AE + Night vs. AE + Day + Night vs. Control vs. Placebo) and a 16-node NMA for protein type (Whey protein vs. Soy vs. Yogurt vs. Mixed protein vs. Milk vs. Red meat vs. Collagen vs. Rice protein vs. Casein vs. Peanut vs. Chicken vs. Bovine colostrum vs. Fish vs. Insect protein vs. Control vs. Placebo). The outcomes of muscle mass included fat-free mass (FFM), lean body mass, skeletal muscle mass, and appendicular lean mass measured by bioimpedance analysis, dual-energy X-ray absorptiometry, anthropometry, and so forth. The outcomes of muscle strength included handgrip strength, bench press strength, squat strength, leg extension strength, leg press strength, and chest press strength. The outcomes of physical performance included timed up-to-go test, 6-min walk test, and gait speed.

The Geometry of the Network

In the networks of timing and type comparisons, each time or type was drawn by a node and randomized comparisons between different timing or types were shown by links between the nodes. The size of nodes reflects the number of participants randomly assigned to each treatment.

Risk of Bias

The risk of bias in included studies was assessed by pairs of independent reviewers (H.H. Zhou and Liao) using the revised Cochrane risk of bias tool for randomized trials (Sterne et al., 2019). Revised Cochrane risk of bias tool for randomized trials is structured in five domains: bias arising from the randomization process, bias due to deviations from intended interventions, bias due to missing outcome data, bias in the measurement of the outcome, and bias in the selection of the reported result. Each domain was judged as "low," "high," or "some concern." The overall bias depends on the lowest judgment in any of the domains.

Statistical Analysis

We performed random-effects network meta-analyses within a frequentist framework. Mean difference (MD) with 95% confidence intervals (CIs) of the change from baseline to final intervention was used to express pooled estimations of outcomes. If no reported standard deviations for changes from baseline to final in each group, methods outlined in the Cochrane Handbook were used (Higgins & Green, 2011). Direct and indirect (and mixed) comparisons were accomplished through the netmeta package of R (Neupane et al., 2014). We used a common heterogeneity variance for each comparison to assess the heterogeneity between studies for each NMA. We evaluated global incoherence and local incoherence in the networks between direct and indirect comparisons using a random-effects design-by-treatment interaction model and the node-splitting method, respectively (Dias et al., 2010; Higgins et al., 2012). We used the surface under the cumulative ranking curve to rank the effectiveness of each treatment (Salanti et al., 2011). The presence of small-study effects bias was assessed using comparison-adjusted funnel plots. We also did a sensitivity analysis by excluding high-risk bias studies.

Production of network plots was done using the network and network graphs packages in Stata (version 16.0). Network meta-analyses were done in R (version 4.2.1) using the netmeta package. We used the Cohens d classification of small (0.2), medium (0.5), and large (0.8) to denote the magnitude of effects (Cohen, 1988). The two-sided p-value <.05 was deemed as statistically significant unless specified.

Certainty of Evidence

The Grading of Recommendation, Assessment, Development, and Evaluation (GRADE) approach was used to assess the certainty of the evidence of main outcomes (Brignardello-Petersen et al., 2018). According to the GRADE guideline, RCTs were initially assigned a ranking of high, and heterogeneities among studies and other factors could downgrade or upgrade the quality of evidence (Brignardello-Petersen et al., 2018). We evaluated the confidence in the findings from the NMA using the Confidence in NMA methodological framework and application based on the GRADE approach (Nikolakopoulou et al., 2020). Discrepancies were resolved through discussion with the senior investigator (Yang).

Summary of More and Less Preferred Treatments

We deaw conclusions from the network meta-analyses using a minimally contextualized framework proposed by the GRADE working group (Brignardello-Petersen et al., 2020). For each outcome, we separated treatments as follows: (a) among the least effective: These interventions showed no difference from placebo, (b) effective: inferior to the most effective, but superior to the least effective, and (c) among the most effective: These interventions were all better than placebo and superior to at least one intervention which was "effective." We then divided all three categories into two groups based on the minimally contextualized framework: (a) high certainty (those with moderate-to-high certainty evidence relative to the placebo) and (b) low certainty (those with low-to-very low certainty evidence relative to the placebo; Brignardello-Petersen et al., 2020).

Results

Selection, Characteristics, and Risk of Bias of Studies

The flowchart of literature searching and study selection was presented in Figure 1. A total of 10,577 relevant articles were yielded through the initial search, of which 175 articles were potentially eligible for assessment. Overall, 116 articles were included in the NMA. Out of the 116 RCTs, 109 investigated the timing of protein supplementation, while 107 RCTs investigated the type of protein supplements. The summary of characteristics of the 116 trials was shown in Table 1, and details of those studies were shown in Table S2 (Supplementary Material [available online]). The studies comprised 4711 participants with a mean age of 46 years, a median intervention duration of 12 weeks, and a mean proportion of women of 36%. The risk of bias was assessed by revised Cochrane risk of bias tool for randomized trials tool and was presented in Figures S1 and S2 (Supplementary Material [available online]). Overall, 73 trials (62.9%) were at low risk of bias and 34 trials (29.3%) were at high risk of bias.

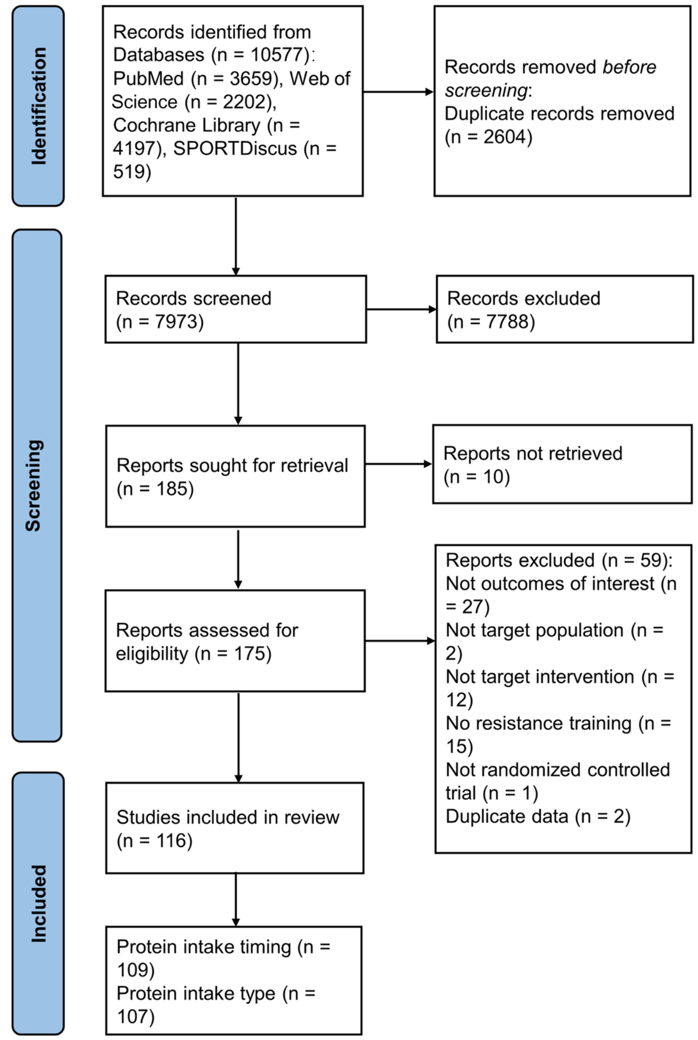

Figure 1 — The flowchart of study selection.

Network Geometry

The overall network plots of protein intake timing and types were shown in Figure 2. For protein timing, a total of 2,443 subjects received protein supplementation. Studied timing was done more commonly AE ($n = 37$ trials, subjects receiving treatment = 860) and during the day ($n = 25$ trials, subjects receiving treatment = 582). Placebo was used as the comparator arm in 79 studies (subjects receiving placebo = 1,318). The most frequently used comparison was protein supplementation AE versus placebo supplementation at the same time ($n = 33$). For protein type, a total of 2,589 subjects received protein supplementation. The studied types were more commonly whey protein ($n = 55$ trials, subjects receiving treatment = 1,046) and milk ($n = 16$ trials, subjects receiving treatment = 358). Placebo was used as the comparator arm in 78 studies (subjects receiving placebo = 1,443). The most frequently used comparison was whey protein versus placebo ($n = 45$). The network plot, the number of studies, and participants for each outcome were shown in Tables S3–S28 (Supplementary Material [available online]).

Certainty of Evidence

The GRADE assessments for all outcomes were presented in the NMA-SoF (summary of finding) tables (Tables S3–S28 in the Supplementary Material [available online]), with the number of included studies and participants, NMA estimates, direct estimates, indirect estimates, the certainty of the evidence, surface under the cumulative ranking curve, and interpretation of findings. Much of the evidence was judged as moderate certainty, rated down most often because of significant imprecision. The NMA results of muscle mass and muscle strength with corresponding GRADE certainty of evidence were shown in Figures 3 and 4, respectively. The NMA results of physical performance were shown in Figure S3 (Supplementary Material [available online]).

Table 1 Summary Characteristics of Included Studies

Characteristics	No. of studies (no. of participants)
Total no. of studies	116 RCTs (timing of protein intake: 108 RCTs; type of protein intake: 107 RCTs)
Total no. of participants	4711
Median (range) no. of participants	30 (12–145)
Mean (*SD*) age (years)	46 (23)
Median (range) intervention length (weeks)	12 (4–72)
Mean female%	36
Outcomes assessed, *n* (%)	
Muscle mass	100 (86)
Muscle strength	78 (67)
Physical performance	23 (20)

Note. RCT = randomized controlled trials.

Network Meta-Analysis

Preference for treatment for all NMA outcomes in comparison with placebo were summarized in Figure 5.

Muscle Mass

Among the timing with moderate certainty evidence relative to placebo, BE + AE proved the most effective protein supplementation timing for FFM (MD: 1.87 [95% CIs 0.84, 2.90]), whereas AE was inferior to the most effective, but superior to the reference for FFM (MD: 0.54 [95% CIs 0.10, 0.99]; Figure 5, Table S29 in the Supplementary Material [available online]). Among the timing with low certainty evidence relative to placebo, AE + Day might be the effective protein supplementation timing for lean body mass (MD: 8.03 [95% CIs 3.09, 12.96]; Figure 5, Table S29 in the Supplementary Material [available online]). Among the timing with moderate certainty evidence relative to placebo, AE was the effective protein supplementation timing for skeletal muscle mass (MD: 0.34 [95% CIs 0.10, 0.58]; Figure 5, Table S30 in the Supplementary Material [available online]). We found no statistically significant difference for appendicular lean mass among different times of protein supplementation (Figure 5, Table S30 in the Supplementary Material [available online]).

Among the protein types with moderate certainty evidence relative to placebo, Collagen (MD: 1.50 [95% CIs 0.39, 2.60]) and Mixed protein (MD: 1.12 [95% CIs 0.12, 2.11]), and Milk (MD: 1.06 [95% CIs 0.49, 1.64]) and Red meat (MD: 1.03 [95% CIs 0.06, 2.00]) were proven as effective protein supplements for FFM and appendicular lean mass, respectively (Figure 5, Table S31 and S32 in the Supplementary Material [available online]). Among the protein types with low certainty evidence relative to placebo, Whey protein might be the effective protein supplement for lean body mass (MD: 2.88 [95% CIs 0.53, 5.23]; Figure 5, Table S31 in the Supplementary Material [available online]). No statistically significant difference was found for skeletal muscle mass among different types of protein supplementation (Figure 5, Table S32 in the Supplementary Material [available online]).

Muscle Strength

Among the timing with moderate or high certainty evidence relative to placebo, Night (MD: 2.85 [95% CIs 0.49, 5.22]), AE + Day + Night (MD: 15.00 [95% CIs 8.54, 21.46]) and BE + AE + Night (MD: 7.51 [95% CIs 2.40, 12.62]), AE + Night (MD: 15.00 [95% CIs 6.01, 23.99]), and Night (MD: 12.12 [95% CIs 3.26, 20.99]) and AE + Day (MD: 14.10 [95% CIs 1.73, 26.48]) were proven as effective protein supplementation timing for handgrip strength, bench press strength, leg extension strength, and leg press strength, respectively (Figure 5, Tables S33–S35 in the Supplementary Material [available online]). Among the timing with low or very low certainty evidence relative to placebo, Day (MD: 1.35 [95% CIs 0.61, 2.10]) and AE + Day + Night (MD: 23.42 [95% CIs 6.43, 40.40]) might be effective protein supplementation timing for handgrip strength and squat strength, respectively (Figure 5, Tables S33 and S34 in the Supplementary Material [available online]). No statistically significant difference in chest press strength was found among protein supplementation at different times (Figure 5, Table S35 in the Supplementary Material [available online]).

Among the protein types with moderate or high certainty evidence relative to placebo, Mixed protein (MD: 2.65 [95% CIs 0.66, 4.64]), Casein (MD: 15.47 [95% CIs 7.89, 23.06]), Whey protein (MD: 9.17 [95% CIs 1.74, 16.60]), Red meat (MD: 18.00 [95% CIs 0.50, 35.50]) and Yogurt (MD: 15.00 [95% CIs: 5.41, 24.59]), and bovine colostrum (MD: 23.56 [95% CIs 1.70, 43.42]) were proved to be effective protein supplements for handgrip strength, bench press strength, squat strength, leg extension strength, and leg press strength, respectively (Figure 5, Table S36–S38 in the Supplementary Material [available online]). Among the protein types with low certainty evidence relative to placebo, Whey protein might be effective for both handgrip strength (MD: 1.26 [95% CIs 0.21, 2.31]) and leg press strength (MD: 6.56 [95% CIs 0.77, 12.35]; Figure 5, Tables S36 and S38 in the Supplementary Material [available online]), while Milk (MD: 7.95 [95% CIs 0.22, 15.68]) might be the effective protein supplement for leg press strength (Figure 5, Table S38 in the Supplementary Material [available online]). We found no statistically significant differences in chest press strength among different types of protein supplementation (Figure 5, Table S38 in the Supplementary Material [available online]).

Physical Performance

No timing or type of protein supplementation showed a statistically significant effect on timed up-to-go test, 6-min walk test, or gait speed (Figure 5, Table S39–S42 in the Supplementary Material [available online]).

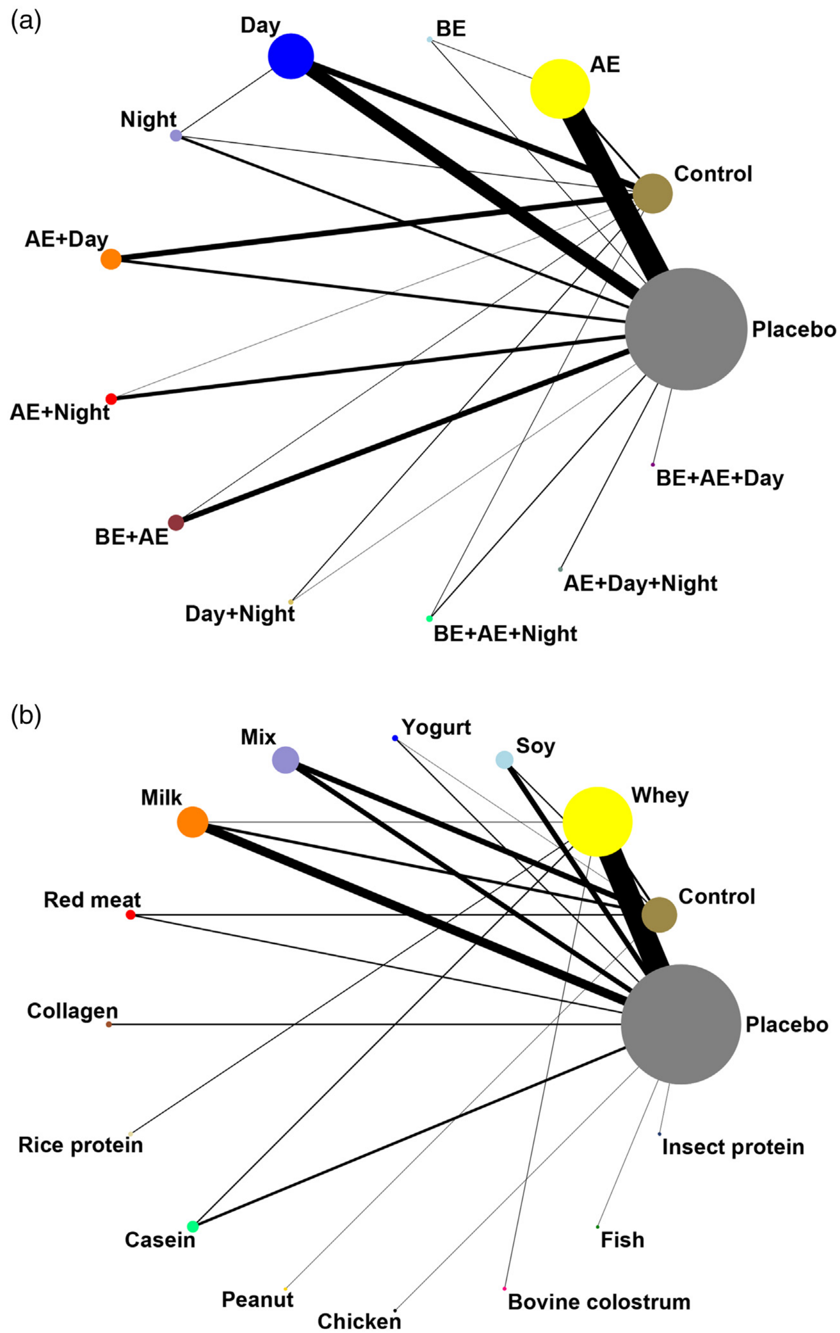

Figure 2 — Network plots of all included studies for protein supplementation timing (a) and type (b). The size of treatment nodes reflects the number of participants randomly assigned to each treatment. The thickness of lines represents the number of studies underlying each comparison. Different shades of the circle represent different interventions. AE = after exercise; BE = before exercise.

Additional Analyses

The results of sensitivity analyses when excluding high-risk bias studies were presented in Table S43–S54 (Supplementary Material [available online]). We did not perform sensitivity analyses for 6-min walk test because of the limited number of eligible studies.

Discussion

This is the first NMA with 116 RCTs to identify effective timing and types of protein supplementation that can be preferably used in improving muscle for subjects performing long-term RT and provide certainty of the evidence for each outcome. Our study revealed that AE and BE + AE were effective protein intake times for the enhancement of muscle mass. Protein supplementation at Night, AE + Day, AE + Night, and BE + AE + Night significantly improved muscle strength with moderate certainty. Furthermore, AE and Night had the most consistent effects on improvement in different outcomes of muscle mass and strength, respectively. Milk, red meat, mixed protein, and collagen had favorable effects on gains of muscle mass. Whey protein, yogurt, casein, bovine colostrum, red meat, and mixed protein could significantly promote

Timing vs. Placebo	FFM (kg)	LBM (kg)	SMM (kg)	ALM (kg)
AE	**0.54 (0.10, 0.99)** [a]	0.34 (–2.20, 2.87) [a]	**0.34 (0.10, 0.58)** [a]	0.35 (–0.17, 0.88) [c]
BE	NA	0.23 (–0.81, 8.53) [a]	0.30 (–0.15, 0.75) [a]	NA
Day	–0.63 (–1.85, 0.59) [b]	0.07 (–2.89, 3.03) [a]	–0.11 (–0.61, 0.40) [b]	0.31 (–0.02, 0.64) [c]
Night	NA	–0.31 (–6.14, 5.53) [b]	NA	0.01 (–0.93, 0.94) [c]
AE + Day	0.05 (–1.42, 1.53) [a]	**8.03 (3.09, 12.96)** [b]	0.17 (–0.52, 0.86) [a]	–0.00 (–1.03, 1.02) [b]
AE + Night	0.27 (–0.94, 1.49) [a]	0.29 (–7.87, 8.46) [b]	–0.20 (–0.40, 0.02) [b]	–0.40 (–1.25, 0.45) [c]
BE + AE	**1.87 (0.84, 2.90)** [a]	0.09 (–4.34, 4.52) [a]	0.43 (–0.02, 0.84) [a]	NA
Day + Night	–0.50 (–2.88, 1.88) [a]	–2.03 (–11.24, 7.18) [a]	NA	NA
BE + AE + Night	NA	–0.04 (–6.90, 6.81) [a]	NA	NA
AE + Day + Night	NA	0.95 (–7.22, 9.12) [a]	NA	NA
BE + AE + Day	NA	NA	NA	NA
Protein vs. Placebo	**FFM (kg)**	**LBM (kg)**	**SMM (kg)**	**ALM (kg)**
Whey	0.42 (–0.19, 1.03) [a]	**2.88 (0.53, 5.23)** [b]	0.11 (–0.10, 0.33) [a]	0.12 (–0.16, 0.41) [b]
Soy	–0.53 (–1.56, 0.50) [a]	–2.06 (–7.98, 3.86) [a]	0.06 (–0.31, 0.44) [a]	–0.20 (–0.87, 0.47) [b]
Yogurt	0.62 (–0.82, 2.06) [a]	0.20 (–8.00, 8.40) [b]	NA	NA
Mix	**1.12 (0.12, 2.11)** [a]	–1.70 (–5.53, 2.12) [b]	0.46 (–0.72, 1.63) [a]	0.41 (–0.44, 1.26) [b]
Milk	1.15 (–0.11, 2,40) [a]	–1.25 (–4.95, 2.45) [a]	0.30 (–1.26, 1.86) [b]	**1.06 (0.49, 1.64)** [a]
Red meat	1.21 (–0.14, 2.55) [a]	–3.06 (–9.25, 3.13) [a]	NA	**1.03 (0.06, 2.00)** [a]
Collagen	**1.50 (0.39, 2.60)** [a]	NA	NA	NA
Rice protein	0.92 (–1.14, 2.98) [a]	2.16 (–6.37, 10.69) [b]	NA	NA
Casein	NA	1.03 (–3.47, 5.52) [a]	NA	NA
Peanut	NA	–4.74 (–12.04, 2.55) [a]	NA	NA
Chicken	NA	0.25 (–6.52, 7.02) [b]	NA	NA
Bovine colostrum	NA	2.96 (–8.99, 14.91) [b]	NA	NA
Fish	NA	NA	–0.10 (–1.22, 1.02) [a]	NA
Insect protein	NA	NA	NA	NA

Moderate certainty	Low certainty	Very low certainty

Figure 3 — Network meta-analysis results of protein supplementation timing and type for muscle mass. Values correspond to mean differences (in kilograms; 95% confidence intervals) in various protein supplementation timing or type and placebo. *a* represents moderate GRADE certainty of the evidence; *b* represents low GRADE certainty of the evidence; *c* represents very low GRADE certainty of the evidence. Values in bold indicate a statistically significant treatment effect. AE = after exercise; ALM = appendicular lean mass; BE = before exercise; FFM = fat-free mass; LBM = lean body mass; SMM = skeletal muscle mass; GRADE = grading of recommendations, assessment, development, and evaluation.

increases in muscle strength, and whey protein had the most consistent effects on different outcomes of muscle strength. Additionally, milk proteins (i.e., milk, whey protein, yogurt, casein, and bovine colostrum), red meat, and mixed protein were effective proteins for gains in both muscle mass and strength. No timing or type of protein supplementation showed a significant enhancement in physical performance.

In contrast to the previous meta-analyses (Schoenfeld et al., 2013; Wirth et al., 2020), our study suggested that AE and Night might be the most beneficial protein intake timing for improving muscle mass and strength, respectively. We included 116 RCTs with 11 nodes of timing, resulting in approximately five times the number of participants included in those two meta-analyses (Schoenfeld et al., 2013; Wirth et al., 2020). Of note, these two meta-analyses explored the effect of protein timing restricted to BE and/or AE versus other times which were not around exercise. This might be inappropriate since other timing, such as night, was found to be beneficial for the enhancement of muscle strength in our present study. Recently, a meta-analysis including 16 RCTs investigated the differences in the effects of protein sources on muscle mass and strength and found animal proteins tend to be more beneficial than plant proteins for muscle mass in adults with and without RT (Lim et al., 2021). Our analysis supported the finding that animal proteins tended to be more effective in enhancing muscle mass and strength than plant proteins since all effective protein types we found were animal-derived proteins.

The strategic consumption of peri-exercise protein has been concerned that it might maximize muscle mass and strength gains. Peri-exercise (BE and AE) protein consumption combined with RT could activate the phosphorylation of mammalian target of rapamycin, the key signaling protein of MPS, thus promoting muscle hypertrophy (Farnfield et al., 2012). Moreover, several studies showed that peri-exercise protein stimulated the intracellular signaling pathways that regulate myofibrillar, mitochondrial protein synthesis, and glycogen synthesis (Breen et al., 2011; Coffey et al., 2011; Ferguson-Stegall et al., 2011). It should also be mentioned

Timing vs. Placebo	Handgrip strength (kg)	Bench press 1RM (kg)	Squat 1RM (kg)	Leg extension 1RM (kg)	Leg press 1RM (kg)	Chest press 1RM (kg)
AE	0.80 (–0.16, 1.76)[b]	0.43 (–1.49, 2.36)[b]	5.18 (–1.44, 11.81)[c]	–3.00 (–7.20, 1.20)[b]	7.05 (–0.93, 15.04)[b]	0.77 (–0.91, 2.45)[c]
BE	NA	0.33 (–5.66, 6.31)[b]	3.18 (–17.90, 24.27)[c]	NA	12.71 (–2.57, 27.99)[b]	NA
Day	**1.35 (0.61, 2.10)**[c]	–0.27 (–2.73, 2.20)[c]	2.74 (–14.18, 19.66)[d]	0.01 (–3.88, 3.91)[b]	0.35 (–5.48, 6.18)[d]	NA
Night	**2.85 (0.49, 5.22)**[b]	–0.17 (–6.86, 6.52)[b]	NA	3.00 (–5.16, 11.16)[b]	**12.12 (3.26, 20.99)**[b]	0.88 (–0.59, 2.21)[d]
AE + Day	0.14 (–0.94, 1.22)[b]	4.76 (–3.10, 12.63)[b]	14.40 (–5.66, 34.46)[c]	–0.41 (–8.94, 8.11)[b]	**14.10 (1.73, 26.48)**[b]	NA
AE + Night	–0.76 (–2.88, 1.35)[c]	–1.53 (–6.36, 3.30)[b]	NA	**15.00 (6.01, 23.99)**[a]	–5.00 (–23.66, 13.66)[b]	NA
BE + AE	NA	1.56 (–0.98, 4.10)[b]	3.23 (–8.90, 15.37)[d]	NA	1.60 (–8.77, 11.97)[b]	NA
Day + Night	NA	–1.22 (–14.65, 12.20)[b]	–5.21 (–35.60, 25.18)[d]	NA	NA	NA
BE + AE + Night	NA	**7.51 (2.40, 12.62)**[b]	10.10 (–2.37, 22.58)[c]	NA	–14.67 (–39.13, 9.79)[b]	NA
AE + Day + Night	NA	**15.00 (8.54, 21.46)**[b]	**23.42 (6.43, 40.40)**[d]	NA	51.00 (–42.67, 144.67)[b]	NA
BE + AE + Day	NA	NA	NA	–4.65 (–10.71, 1.41)[b]	NA	NA
Protein vs. Placebo	**Handgrip strength (kg)**	**Bench press 1RM (kg)**	**Squat 1RM (kg)**	**Leg extension 1RM (kg)**	**Leg press 1RM (kg)**	**Chest press 1RM (kg)**
Whey	**1.26 (0.21, 2.31)**[c]	2.39 (–0.06, 4.83)[b]	**9.17 (1.74, 16.60)**[b]	–4.66 (–9.80, 0.47)[b]	**6.56 (0.77, 12.35)**[c]	0.41 (–0.91, 1.72)[c]
Soy	0.26 (–1.58, 2.09)[c]	0.69 (–3.46, 4.84)[b]	3.60 (–8.00, 15.17)[b]	NA	6.38 (–21.05, 8.29)[c]	NA
Yogurt	NA	1.30 (–10.75, 8.15)[b]	–1.60 (–21.00, 17.80)[b]	**15.00 (5.41, 24.59)**[a]	NA	NA
Mix	**2.65 (0.66, 4.64)**[b]	0.34 (–3.97, 3.30)[b]	9.45 (–9.22, 28.13)[b]	NA	3.60 (–3.74, 10.93)[b]	NA
Milk	0.49 (–0.60, 1.58)[b]	4.42 (–1.68, 10.52)[b]	NA	–0.68 (–4.42, 3.07)[b]	**7.95 (0.22, 15.68)**[c]	–0.30 (–6.35, 5.75)[b]
Red meat	NA	–0.01 (–9.21, 9.20)[b]	1.13 (–25.86, 28.12)[b]	**18.00 (0.50, 35.50)**[b]	–20.09 (–57.39, 17.21)[c]	NA
Collagen	1.40 (–1.09, 3.89)[b]	2.09 (–5.47, 9.65)[b]	10.32 (–4.09, 24.73)[b]	NA	NA	NA
Rice protein	NA	2.99 (–6.99, 12.96)[b]	NA	NA	–6.84 (–24.11, 10.44)[b]	NA
Casein	0.00 (–2.57, 2.57)[b]	**15.47 (7.89, 23.06)**[a]	–13.93 (–34.81, 6.95)[b]	–0.76 (–7.10, 5.59)[b]	10.34 (–0.32, 20.99)[b]	2.03 (0.08, 3.97)[c]
Peanut	NA	NA	NA	NA	NA	NA
Chicken	NA	5.00 (–14.16, 24.16)[b]	NA	NA	NA	NA
Bovine colostrum	NA	–1.62 (–14.67, 11.44)[b]	NA	NA	**23.56 (1.70, 43.42)**[b]	NA
Fish	NA	NA	NA	NA	–0.10 (–19.50, 19.30)[b]	NA
Insect protein	NA	–6.00 (–16.04, 4.04)[b]	NA	NA	–5.00 (–23.54, 13.54)[b]	NA

High certainty | Moderate certainty | Low certainty | Very low certainty

Figure 4 — Network meta-analysis results of protein supplementation timing and type for muscle strength. *Note*. Values correspond to mean differences (in kilograms; 95% confidence intervals) in various protein supplementation timing or type and placebo. *a* represents high GRADE certainty of the evidence; *b* represents moderate GRADE certainty of the evidence; *c* represents low GRADE certainty of the evidence; *d* represents very low GRADE certainty of the evidence. Values in bold indicate a statistically significant treatment effect. AE = after exercise; BE = before exercise; 1RM = one-repetition maximum; GRADE = grading of recommendations, assessment, development, and evaluation.

that the majority of studies that examined postexercise protein intake administered the same dose of protein BE as well (Herda et al., 2013; Lockwood et al., 2017; Nabuco et al., 2018; Obradović et al., 2020; Ozan et al., 2020; Park et al., 2019; Spillane & Willoughby, 2016; Taylor et al., 2016). Therefore, it cannot be ruled out that supplementing protein before exercise is an important time point in seeking to optimize muscle size and strength gain. Our study combined direct and indirect evidence and examined that postexercise might be a more consistent effective timing than preexercise for muscle mass gains. However, this finding requires caution as only one study (Schoenfeld et al., 2017) directly compared preexercise protein feeding with postexercise protein feeding. Future studies that directly investigate the comparative effect of protein delivered preexercise versus postexercise under different physical conditions are needed to confirm this finding.

Researchers have illustrated that Night protein intake with or without RT could lead to acute increases in MPS and improve postexercise overnight recovery (Holwerda et al., 2016; Kouw et al., 2017; Res et al., 2012; Trommelen et al., 2016). Our study, which included RCTs with a median duration of 12 weeks of protein intervention, suggests that the acute increase of MPS rate activated by Night protein supplementation might translate to longitudinal changes in strength or fiber composition of muscle, but not hypertrophy. While eating at night or before sleep has always been controversial, growing evidence exists to support the benefits of protein consumption before sleep. It was found that the combination of presleep protein supplementation and exercise reduced blood pressure and arterial stiffness in obese women with high blood pressure (Figueroa et al., 2014). Furthermore, Ormsbee et al. (2015) also concluded that 4 weeks of Night protein feeding combined with exercise training did not impact insulin sensitivity in obese women.

It was demonstrated that there exists a "muscle full" effect that muscle responses to continual protein feeding are transient (Atherton et al., 2010; Millward, 1995). Atherton et al. (2010) and Atherton and Smith (2012) using [1, 2-$^{13}C_2$] leucine for determination of MPS and anabolic signaling found that despite sustained amino acids, the MPS rate peaked and then returned to baseline within 90 min of protein feeding. In our present study, in addition to the peri-exercise period and Night, other effective protein intake times were all combinations of multiple time points (such as AE + Day + Night, BE + AE + Night). Therefore, our study also suggests that protein intake evenly at different effective times might be a more effective strategy than at one single time for muscle mass and strength gains.

The isolated animal-source protein, with a sufficient quantity of essential amino acids (EAAs) and fast digestible characteristics, is generally considered superior to the isolated vegetarian protein

Timing vs. Placebo	FFM (kg)	LBM (kg)	SMM (kg)	ALM (kg)	Handgrip strength (kg)	Bench press 1RM (kg)	Squat 1RM (kg)	Leg extension 1RM (kg)	Leg press 1RM (kg)	Chest press 1RM (kg)	TUG (s)	6MWT (m)	Gait speed (m / s)
AE	**0.54** [b]	0.34 [e]	**0.34** [b]	0.35 [d]	0.80 [e]	0.43 [e]	5.18 [d]	–3.00 [e]	7.05 [e]	0.77 [d]	–0.25 [e]	–16.60 [d]	–0.13 [d]
BE	NA	0.23 [e]	0.30 [e]	NA	NA	0.33 [e]	3.18 [d]	NA	12.71 [e]	NA	NA	NA	NA
Day	–0.63 [d]	0.07 [e]	–0.11 [d]	0.31 [d]	**1.35** [c]	–0.27 [d]	2.74 [d]	0.01 [e]	0.35 [d]	NA	0.06 [d]	–8.85 [d]	0.23 [d]
Night	NA	–0.31 [d]	NA	0.01 [d]	**2.85** [b]	–0.17 [e]	NA	3.00 [e]	**12.12** [b]	0.88 [d]	0.79 [e]	NA	–0.38 [d]
AE + Day	0.05 [e]	**8.03** [c]	0.17 [e]	–0.00 [d]	0.14 [e]	4.76 [e]	14.40 [d]	–0.41 [e]	**14.10** [b]	NA	–0.19 [e]	21.33 [d]	–0.36 [e]
AE + Night	0.27 [e]	0.29 [d]	–0.20 [d]	–0.40 [d]	–0.76 [d]	–1.53 [e]	NA	**15.00** [b]	–5.00 [e]	NA	NA	NA	NA
BE + AE	**1.87** [a]	0.09 [e]	0.43 [e]	NA	NA	1.56 [e]	3.23 [d]	NA	1.60 [e]	NA	NA	NA	NA
Day + Night	–0.50 [e]	–2.03 [e]	NA	NA	NA	–1.22 [e]	–5.21 [d]	NA	NA	NA	NA	NA	–0.29 [e]
BE + AE + Night	NA	–0.04 [e]	NA	NA	NA	**7.51** [b]	10.10 [d]	NA	–14.67 [e]	NA	NA	NA	NA
AE + Day + Night	NA	0.95 [e]	NA	NA	NA	**15.00** [b]	**23.42** [c]	NA	51.00 [e]	NA	NA	NA	0.00 [e]
BE + AE + Day	NA	NA	NA	NA	NA	NA	NA	–4.65 [e]	NA	NA	NA	NA	NA
Protein vs. Placebo	**FFM (kg)**	**LBM (kg)**	**SMM (kg)**	**ALM (kg)**	**Handgrip strength (kg)**	**Bench press 1RM (kg)**	**Squat 1RM (kg)**	**Leg extension 1RM (kg)**	**Leg press 1RM (kg)**	**Chest press 1RM (kg)**	**TUG (s)**	**6MWT (m)**	**Gait speed (m / s)**
Whey	0.42 [e]	**2.88** [c]	0.11 [e]	0.12 [d]	**1.26** [c]	2.39 [e]	**9.17** [b]	–4.66 [e]	**6.56** [c]	0.41 [d]	–0.05 [d]	–22.79 [d]	–0.02 [e]
Soy	–0.53 [e]	–2.06 [e]	0.06 [e]	–0.20 [d]	0.26 [d]	0.69 [e]	3.60 [e]	NA	6.38 [d]	NA	–0.32 [d]	–25.24 [d]	0.10 [d]
Yogurt	0.62 [e]	0.20 [d]	NA	NA	NA	1.30 [e]	–1.60 [e]	**15.00** [b]	NA	NA	NA	NA	NA
Mix	**1.12** [b]	–1.70 [d]	0.46 [e]	0.41 [d]	**2.65** [b]	0.34 [e]	9.45 [e]	NA	3.60 [e]	NA	–0.25 [e]	8.80 [d]	0.43 [e]
Milk	1.15 [e]	–1.25 [e]	0.30 [d]	**1.06** [b]	0.49 [e]	4.42 [e]	NA	–0.68 [e]	**7.95** [c]	–0.30 [e]	–0.09 [e]	–13.00 [e]	–0.03 [e]
Red meat	1.21 [e]	–3.06 [e]	NA	**1.03** [b]	NA	–0.01 [e]	1.13 [e]	**18.00** [b]	–20.09 [d]	NA	–0.49 [e]	NA	0.00 [e]
Collagen	**1.50** [b]	NA	NA	NA	1.40 [e]	2.09 [e]	10.32 [e]	NA	NA	NA	NA	NA	NA
Rice protein	0.92 [e]	2.16 [d]	NA	NA	NA	2.99 [e]	NA	NA	–6.84 [e]	NA	NA	NA	NA
Casein	NA	1.03 [e]	NA	NA	0.00 [e]	**15.47** [b]	–13.93 [e]	–0.76 [e]	10.34 [e]	2.03 [d]	–0.23 [e]	NA	NA
Peanut	NA	–4.74 [e]	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Chicken	NA	0.25 [d]	NA	NA	NA	5.00 [e]	NA	NA	NA	NA	NA	NA	NA
Bovine colostrum	NA	2.96 [d]	NA	NA	NA	–1.62 [e]	NA	NA	**23.56** [b]	NA	NA	NA	NA
Fish	NA	NA	–0.10 [e]	NA	NA	NA	NA	NA	–0.10 [e]	NA	NA	NA	NA
Insect protein	NA	NA	NA	NA	NA	–6.00 [e]	NA	NA	–5.00 [e]	NA	NA	NA	NA

- Among the most effective: "Superior to at least one effective"with moderate to high certainty
- Effective: "Inferior to the most effective/superior to the reference" with moderate to high certainty
- Maybe among most effective: "Superior to at least one effective" with very low to low certainty
- Maybe effective: "Inferior to the most effective/superior to the reference" with very low to low certainty
- Maybe no effective: "Maybe among the least effective" with very low to low certainty
- No effective: "Among the least effective" with moderate to high certainty

Figure 5 — Summary results of protein supplementation timing and type network meta-analysis for all outcomes. The number is the point estimate of effect in comparison with placebo. Values in bold indicate a statistically significant treatment effect. *a* represents among the most effective: "Superior to at least one effective" with moderate to high certainty; *b* represents effective: "Inferior to the most effective/superior to the reference" with moderate to high certainty; *c* represents maybe effective: "Inferior to the most effective/superior to the reference" with very low to low certainty; *d* represents maybe no effective: "Maybe among the least effective" with very low to low certainty; *e* represents no effective: "Among the least effective" with moderate to high certainty. AE = after exercise; ALM = appendicular lean mass; BE = before exercise; FFM = fat-free mass; LBM = lean body mass; SMM = skeletal muscle mass; TUG = timed up-to-go test; 1RM = one-repetition maximum; 6MWT = 6-min walk test.

for protein synthesis stimulation (Deldicque, 2020; van Vliet et al., 2015). The duration of postprandial MPS appears to be limited by leucine concentration, ATP status, and the availability of EAAs (Churchward-Venne et al., 2012). Plant proteins are more likely to be used for urea synthesis rather than muscle building due to the lack of specific EAAs (Gilbert et al., 2011; van Vliet et al., 2015). Whey protein and casein, both the main components of milk proteins, are rich in leucine and other EAAs. However, due to the fast-digesting properties of whey protein, it appears to be more effective at increasing MPS. Most current studies also demonstrated that isolated or mixed milk proteins tend to optimally facilitate MPS compared with other proteins (Reitelseder et al., 2011; Tang et al., 2009; Wilkinson et al., 2007).

Our study has several strengths. First, we used the method of NMA, which could summarize both direct and indirect comparisons to provide more robust evidence than conventional pairwise meta-analyses (Nikolakopoulou et al., 2018; Petropoulou et al., 2017). Second, we conducted a comprehensive literature search to include different timing or types of protein supplementation, thus, we could innovatively compare the effectiveness of different timing or types of protein supplementation on muscle mass, strength, and physical performance. Last, we rated the certainty of the evidence for each outcome using the GRADE approach and applied a minimally contextualized framework to summarize the results transparently and efficiently.

Several limitations need to be addressed. First, many of our results lacked support from direct comparative evidence. However, given the methodological strength of NMA (Nikolakopoulou et al., 2018), we believe our results could provide meaningful insights until further studies establish stronger evidence. Second, we only examined the effects of protein intake in adults undergoing RT; thus, it remains unclear the impact of the timing or type of protein supplementation in adults receiving endurance exercise training or without exercise training. Third, studies examining timing effects of protein ingestion often fail to rigorously equate total daily protein intake in the control group. Therefore, compared with the control group, the "protein timing" group has an inherent advantage of a larger daily total protein intake. Finally, among the factors that enhance muscle adaptation to RT, the total daily protein content is more important than time (Schoenfeld et al., 2013). Therefore, caution is necessary when interpreting studies aimed at investigating protein timing.

Conclusions

Overall, pre/postexercise and at night are the key recommended times of protein intake to increase muscle mass and strength, respectively. Animal-source proteins, especially milk proteins, are the preferred types of protein supplements for the improvement of muscle mass and strength. Given the large uncertainty in comparative estimates, future RCTs that directly compare the effects of different timing or types of protein supplementation on muscle mass, strength, and physical performance are of great requirement.

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