Effect of whey protein supplementation on body composition changes in women: a systematic review and meta-analysis

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Context: *A preponderance of evidence supports the beneficial effects of whey protein (WP) supplementation on body composition in men; however, there is currently insufficient evidence to make an equivalent claim in women.* ***Objective:*** *This systematic review and meta-analysis assessed the effects of WP supplementation with or without energy restriction (ER) and resistance training (RT) on changes in body mass, lean mass, and fat mass in women.* ***Data Sources:*** *Pubmed, Scopus, Cochrane, and CINAHL were searched using the keywords "whey protein," "body composition," and "lean mass."* ***Data Extraction:*** *Two researchers independently screened 1845 abstracts and extracted 276 articles. Thirteen randomized controlled trials with 28 groups met the inclusion criteria.* ***Results:*** *Globally, WP supplementation increased lean mass (WMD, 0.37 kg; 95% confidence interval [CI], 0.06 to 0.67) while not influencing changes in fat mass (−0.20 kg; 95%CI, −0.67 to 0.27) relative to non-WP control. The beneficial effect of WP on lean mass was lost when only studies with RT were included in the analysis (n = 7 comparisons; 0.23 kg; 95%CI, −0.17 to 0.63). The beneficial effect of WP on lean mass was more robust when only studies with an ER component were included (n = 6 comparisons; 0.90 kg; 95%CI, 0.31 to 1.49). There was no effect of WP on lean mass in studies without ER (n = 9 comparisons; 0.22 kg; 95%CI, −0.12 to 0.57).* ***Conclusion:*** *Whey protein supplementation improves body composition by modestly increasing lean mass without influencing changes in fat mass. Body composition improvements from WP are more robust when combined with ER .*

INTRODUCTION

A preponderance of evidence indicates that higher-protein diets are an effective means to improve body composition in various energy states and exercise training conditions.[1–3] Protein supplementation is one dietary strategy that can help individuals attain higher total daily protein intake. Protein supplementation is promoted as a means to help individuals improve their body composition, especially when consumed in conjunction with weight loss and (or) exercise training. Whey protein (WP) may be a particularly effective form of protein supplementation to increase muscle protein synthesis[4,5] because of its rapid absorption kinetics[6] and high concentration of branched-chain amino acids.[7,8] Indeed, WP may be an optimal protein source to support lean body mass gains.[8,9]

Despite discordance among individual studies, recent systematic reviews and meta-analyses tend to indicate that protein supplementation favors modest increases in lean mass.[10–12] Although the training history[11] and age[3,13] of study participants were investigated as potential mediators in the relationship between protein supplementation and changes in body composition,

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Table 1 **PICOS criteria for inclusion and exclusion of studies**

Parameter	Description
Population	Adult women, mean age $\geq$19 y
Intervention	a. Groups with purposeful diet-induced energy restriction undergoing resistance training protocol and whey protein supplementation b. Groups with purposeful diet-induced energy restriction and whey protein supplementation c. Groups in energy balance (without purposeful weight loss or weight gain) undergoing resistance training protocol and whey protein supplementation d. Groups in energy balance (without purposeful weight loss or weight gain) consuming a whey protein supplement
Comparison	a. Groups with purposeful diet-induced energy restriction undergoing resistance training protocol without whey protein supplementation b. Groups with purposeful diet-induced energy restriction without whey protein supplementation c. Groups in energy balance (without purposeful weight loss or weight gain) undergoing resistance training protocol without whey protein supplementation d. Groups in energy balance (without purposeful weight loss or weight gain) consuming a whey protein supplement
Outcome	Changes in whole-body composition, including body mass, lean mass, and fat mass
Setting	Randomized controlled trials
Research Question	What is the effect of whey protein supplementation on whole-body composition in women (with or without energy restriction and with or without resistance training)?

relatively little attention has been paid to potential sex differences in body composition responses. Notably, females are underrepresented in this line of research, as evinced by male-only populations in 15 of 22 studies in the most-cited protein supplementation meta-analysis.[12] Of practical concern, there is a public perception that WP supplementation will lead to excessive hypertrophy or "bulkiness" in women (unpublished data, 2014 Consumer Whey Protein Tracking Study). Therefore, the purpose of the present systematic review and meta-analysis of randomized control trials was to assess the effect of WP supplementation on body composition changes over time in adult women. It was hypothesized that, globally, WP supplementation would moderately improve body composition but would not cause excessive muscle hypertrophy. This investigation was conducted in a 2×2 factorial manner with a priori subgroup analyses to assess whether this effect is demonstrable during weight stability 1) with and 2) without resistance training (RT) and during diet-induced weight loss (WL) 3) with and 4) without RT.

METHODS

This systematic review and meta-analysis was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines.[14] The procedures for identification, screening, data extraction, and analysis were agreed upon in advance among all authors. The population, intervention, comparison, outcome, and setting (PICOS) criteria were used to define the research questions (Table 1).

Data sources

A systematic search of literature was conducted independently by 2 reviewers in January 2017, and is current to August 2017. The following databases were searched: PubMed, Cochrane, Scopus, and CINAHL. The combinations of keywords and specific search parameters can be found in Table S1 in the Supporting Information online. Additionally, manual searches and reference lists of previous protein supplementation reviews were used for identification of articles.

Inclusion and exclusion criteria

Published randomized controlled trials that included WP supplementation were considered for this systematic review and meta-analysis. Study population was limited to apparently healthy (not characterized as having a specific chronic disease), nonpregnant females aged $\geq$ 19 years. In addition to studies with only female participants, studies with both male and female participants were included if data on primary outcome measures were available specifically for female participants in the manuscript (or if data were able to be obtained by contacting authors). Interventions had to be of parallel design and of at least 6 weeks duration. The treatment group (WP supplementation) had to be contrasted with an isocaloric non-WP control. Multi-ingredient supplements were acceptable if the only difference between the treatment and control was WP (eg, WP + carbohydrate + calcium vs carbohydrate + calcium). Whey protein concentrates, isolates, and hydrosylates were considered acceptable forms of supplementation, but the supplement could not include

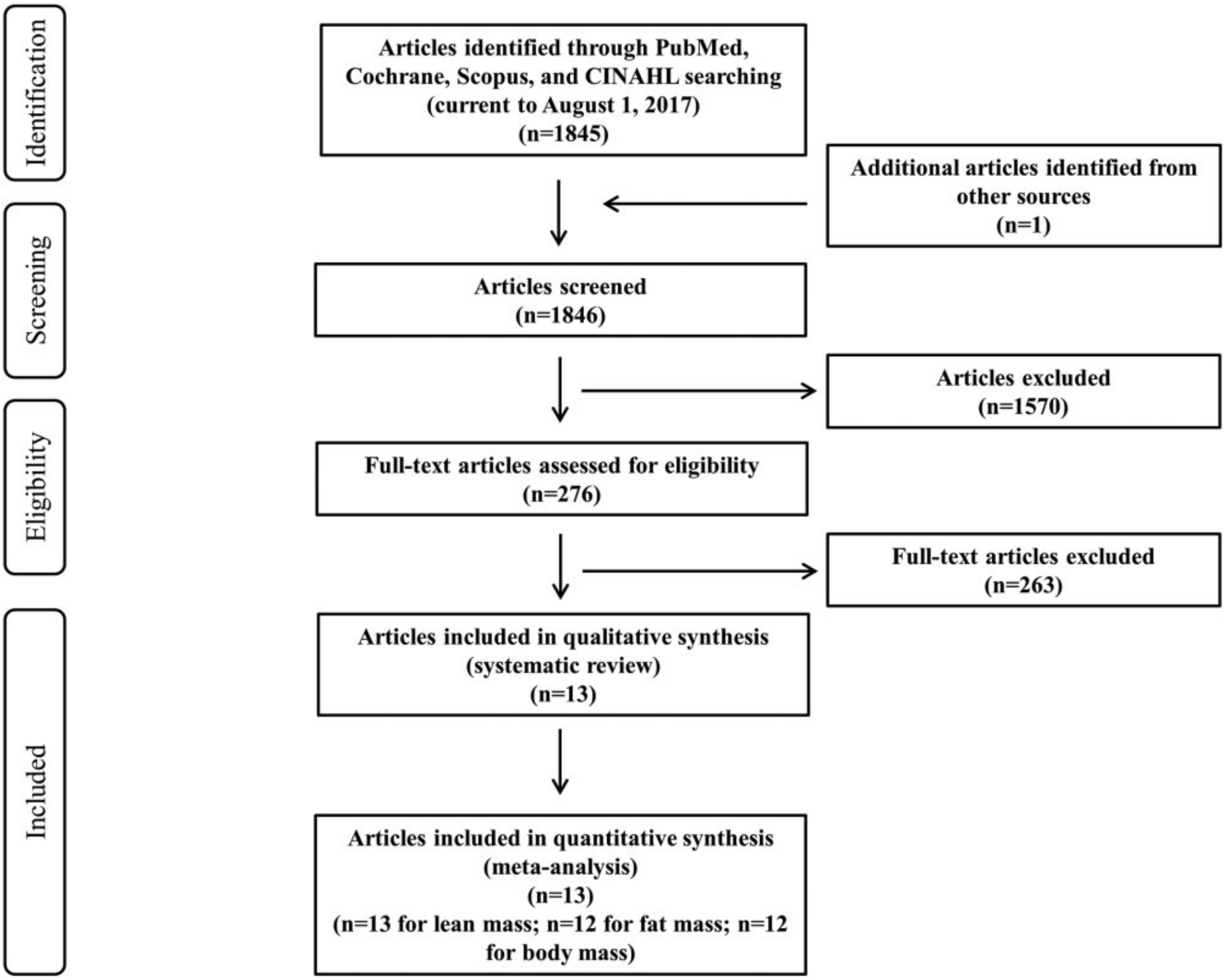

Figure 1 **Flow diagram of the literature search process.**

other types of protein (eg, casein). Acceptable forms of body composition measurement included dual-energy X-ray absorptiometry, air-displacement plethysmography, and hydrostatic weighing. Interventions involving very low calorie diets (<800 kcal/d) were excluded. There was no lower limit for publication date.

Article identification and data extraction

A multipass method was used to identify relevant studies from the 1845 articles captured in the original search (Figure 1). The first pass involved independently screening titles and abstracts to determine whether studies met inclusion criteria. If the abstract did not provide sufficient information to categorically exclude the article, the entire article was retrieved for review in the next pass. After each pass, a cross-check was performed and differences between reviewers were discussed and reconciled. One thousand five hundred sixty-nine studies were excluded from the first pass, leaving 276 articles for full-text review in the second pass. Two hundred thirty-eight articles were excluded following full-text review for the following reasons: participant group had a mean age <19 years, study participants were characterized as having a chronic disease or severe injury, study used an unacceptable method of body composition assessment, primary outcomes were not reported or only reported graphically (or if reported, most often group means for men + women, but data only on women not available), leaving 38 articles for potential inclusion in qualitative and quantitative analysis. Notably, studies with both sexes were retained as part of these 38 articles.

Complete data for inclusion in the meta-analyses were available in 4 articles.[15–18] Corresponding authors of the remaining 34 articles were contacted via email to acquire unpublished data (most often to acquire female-only data). Authors of 9 articles[19–27] provided data via email, which permitted inclusion in the meta-analysis. Twenty-four articles were not included in the final analysis when additional information led to discovery that casein was included in the WP supplement (n = 1), the original data were lost (n = 1), or primary outcome data were unavailable (n = 22).

When individual studies included multiple groups that would classify as an intervention group (eg, different WP doses), each was treated as a distinct intervention. When studies included multiple groups that would classify as a comparator group (eg, soy and carbohydrate separately compared with WP), only the carbohydrate control group was included. Change value (Δ) means and standard deviations (SDs) for each primary outcome (lean mass, fat mass, body mass) were extracted when available. Otherwise, Δmean and ΔSD were calculated from pre- and postintervention values when raw data were provided. When ΔSD was not available, the correlation cofactor (corr) was calculated from studies in which pre-SD, post-SD, and ΔSD were available, as described previously.[28]

The following data were extracted from selected articles independently by both reviewers: author's last name; publication year; title; body composition assessment method; sample size of each intervention group; mean age of participants; intervention duration; exercise characteristics and modality; intervention supplement characteristics; WP supplementation dose (g/d and g/kg/d); number of WP supplementation doses per day; amount of WP per supplementation dose; total protein intake (g/d and g/kg/d); energy deficit; techniques for dietary control and monitoring compliance; pre- and post-intervention and net changes in body mass, lean mass, and fat mass; and term used to define lean mass.

Assessment of risk of bias

The risks of selection, performance, and detection biases were evaluated from included studies using a modified Cochrane tool for assessing risk of bias (Table 2).[15–27] Both reviewers independently assessed risk of bias by scoring domains of selection bias, performance bias, and detection bias as "high risk," "low risk," or "unclear risk" of bias.

Statistical analysis

Statistical analyses were conducted on Stata/SE 12 software (StataCorp LP, College Station, TX, USA), and results are reported as mean ± SD or weighted mean differences WMD and 95% confidence intervals (CI). The overall effect sizes were calculated using the Stata 12 metaan function, using either the fixed-effects or random-effects option, depending on heterogeneity statistics. Heterogeneity was assessed using χ^2 tests and the I^2 statistic. A significant χ^2 test ($P < 0.05$) and an I^2 statistic of >50% indicated heterogeneity in effect sizes among the studies and therefore warranted the use of a random-effects model. When the χ^2 test was nonsignificant ($P \geq 0.05$) and the I^2 statistic was less than 50%, a fixed-effects model was used.

Sensitivity analyses were performed by removing each study one by one and repeating the analysis. Thirteen studies[15–27] contributing 15 comparisons were included in the primary meta-analysis. Removal of any individual study, with the exception of that by Weinheimer et al.,[15] did not influence results or degree of heterogeneity. Variation in point estimates attributable to heterogeneity (I^2) is largely a function of sample size in individual studies.[29] Removal of large studies (even those near the middle of the distribution of effect sizes, such as Weinheimer et al.[15]) (Figure 2[15–27]) can increase within-study precision and reduce I^2 despite the removal potentially increasing heterogeneity.[29] Weinheimer et al.[15] contributes approximately 33% weight of the primary meta-analysis. Given this weight (and markedly smaller confidence intervals than other comparisons), any failure of confidence intervals overlapping would indicate an "inconsistency" with other studies from metrics used to assess heterogeneity. Weinheimer et al.[15] was retained in the primary meta-analysis given these considerations and the fact that eligibility criteria were sound and data were correct.

Each specific objective (effect of WP on body composition with or without ER and with or without RT) is presented as a subgroup analysis. Secondary subgroup analyses were of WP versus a carbohydrate control independent of ER and RT status; WP versus control with and without RT separately (independent of ER); and WP versus control with and without ER separately (independent of RT).

RESULTS

Study features and participant characteristics

Thirteen articles[15–27] that met all inclusion criteria contributed 28 intervention groups (488 female participants) resulting in 15 WP versus control comparisons. Two articles each contributed 2 intervention comparisons to a control.[15,20] Descriptions of study features and participant characteristics are summarized in Table 3.[15–27] Concerning the original 2 × 2 factorial design, 1 comparison met criteria for ER + RT,[23] 6 comparisons were classified as No ER + RT,[15,19,25–27] 5 comparisons were ER + No RT,[16–18,21,24] and 3 comparisons were No ER + No RT.[20,22] Publication dates ranged from 2008 to 2017, and intervention durations ranged from 6 weeks to 12 months. Among the 28 intervention groups, mean ages ranged from 20 ± 2 years to

Table 2 **Risk of bias assessment of randomized controlled trials included in a meta-analysis on the effects of consuming a whey-protein supplement on body composition in women**

References	Selection bias		Performance bias	Detection bias	Dietary control		Body composition assessment method
	Randomization	Allocation concealment	Blinding of participants and study investigator	Blinding of outcome assessment	Dietary prescription	Dietary compliance	
Adechian et al. (2012)[24]	Unclear	Unclear	Unclear	Unclear	Personalized menus to provide energy equal to the basal energy requirements; provided supplement	—	DXA
Duff et al. (2014)[25]	Low risk	Unclear	Low risk	Unclear	Provided supplement	Supplement log; returned excess supplement	DXA
Gordon et al. (2008)[18]	Unclear	Unclear	Unclear	Unclear	Diets developed to elicit 400 kcal/d energy deficit; provided lunch, dinner, and snack meals; provided supplement to high-protein group	—	DXA
Holm et al. (2008)[27]	Unclear	Unclear	Low risk	Unclear	Provided supplement	Completed 4-d weighed food record at 0, 12, and 24 wk	DXA
Keogh and Clifton (2008)[21]	Low risk	Unclear	Low risk	Unclear	Provided supplement; advised to replace 2 meals/d with protein supplement	Compliance assessed by daily checklist and participant returning empty satchets	DXA
Kjølbæk et al. (2017)[20]	Low risk	Low risk	Low risk	Low risk	Monthly dietary counseling sessions; provided supplement	Completed 3-d food records at baseline, 6 wk, and upon completion	DXA
Martens et al. (2015)[22]	Unclear	Unclear	Low risk	Unclear	Personalized menus/recipes to maintain body weight; provided supplement	Dietary compliance monitored at weeks 5 and 9 via interim visits to facility and interview	Bod Pod
Mojtahedi et al. (2011)[17]	Low risk	Low risk	Low risk	Low risk	Diet education classes every 2 wk; diets developed to elicit 500 kcal/d energy deficit; provided supplement	Completed 3-d food diaries at baseline, month 3, and postintervention; used supplement containers collected and weighed	DXA
Stragier et al. (2016)[26]	Unclear	Unclear	Unclear	Unclear	Provided supplement; instructed to maintain normal eating pattern	Completed food survey over 7 continuous days; nitrogen balance determined	—
Sukumar et al. (2011)[16]	Low risk	Unclear	Unclear	Unclear	Diet developed to elicit 500 kcal/d energy deficit; 36 weight-loss counseling sessions; provided supplement	Completed food records for at least 1 wk each month; FFQ and 24-h recall every 3 mo; protein intake compliance monitored via BUN	DXA
Taylor et al. (2016)[19]	Low risk	Unclear	Unclear	Unclear	Maintain normal dietary intake; provided supplement	Consumed supplement under supervision	DXA
Verreijen et al. (2015)[23]	Low risk	Low risk	Low risk	Low risk	Dietary counseling session every 2 wk; diets developed to elicit 600 kcal/d energy deficit; provided supplement	Completed 3-d food records at baseline and after 7 and 13 wk of intervention and checked for completeness; recorded supplement intake in a diary	DXA
Weinheimer et al. (2012)[15]	Unclear	Unclear	Low risk	Unclear	Provided supplement	Completed 4-d food records at 0, 18, and 36 wk; measured UUR; completed daily supplement logs	DXA

Abbreviations: BUN, blood urea nitrogen; DXA, dual-energy X-ray absorptiometry; FFQ, food frequency questionnaire; UUR, urine urea nitrogen.

References	Whey Protein Mean (kg)	Whey Protein SD (kg)	Whey Protein Participants (no.)	Control Mean (kg)	Control SD (kg)	Control Participants (no.)	Weight (%)	Mean difference (kg), random-effects (95% CI)
Adechian et al (2012)[24]	-3.0	1.6	17	-4.0	2.1	15	4.1	1.00 (-0.31 to 2.31)
Duff et al (2014)[25]	0.5	1.8	13	0.7	2.8	12	2.3	-0.20 (-2.06 to 1.66)
Gordon et al (2008)[18]	-2.1	1.8	9	-4.1	2	15	3.1	2.00 (0.45 to 3.55)
Holm et al (2008)[27]	0.8	1.2	13	0.5	1.3	16	6.7	0.30 (-0.61 to 1.21)
Keogh and Clifton (2008)[21]	-1.5	2.3	11	-2.4	2.1	9	2.2	0.90 (-1.03 to 2.83)
Kjølbæk et al (2017)[20] -Ca	1.9	1.5	31	1.7	1.2	38	9.5	0.20 (-0.45 to 0.85)
Kjølbæk et al (2017)[20]	1.9	1.4	32	1.7	1.2	38	9.9	0.20 (-0.42 to 0.82)
Martens et al (2015)[22]	0.3	4.1	8	-0.3	4.1	10	0.6	0.60 (-3.24 to 4.44)
Mojtahedi et al (2011)[17]	2.0	2.3	13	0.6	1.5	13	3.3	1.38 (-0.11 to 2.87)
Stragier et al (2016)[26]	1.0	0.4	7	1.2	0.5	7	12.4	-0.23 (-0.67 to 0.21)
Sukumar et al (2011)[16]	-1.2	2.5	26	-1.4	2.2	21	4.1	0.20 (-1.12 to 1.52)
Taylor et al (2016)[19]	1.6	1.0	8	1.4	1.3	8	5.0	0.20 (-0.94 to 1.34)
Verreijen et al (2015)[23]	0	1.8	16	-0.3	2.0	16	4.0	0.30 (-1.04 to 1.64)
Weinheimer et al (2012)[15]-20g	1.3	0.2	41	0.6	0.2	40	16.4	0.71 (0.62 to 0.80)
Weinheimer et al (2012)[15]-40g	0.7	0.3	29	0.6	0.2	40	16.3	0.13 (0.02 to 0.24)
Total							100	0.37 (0.06 to 0.67)

Heterogeneity: $Tau^2 = 0.145$; $Chi^2 = 80.13$, df = 14 (P = 0.000); $I^2 = 83\%$

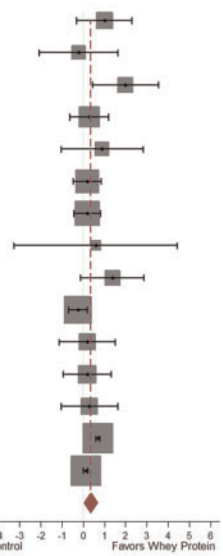

Figure 2 **Effect of whey protein supplementation on changes in lean mass in women.** A random-effects model was used for lean mass because heterogeneity was observed in pooled data. *Abbreviations*: Ca, calcium; CI, confidence interval; SD, standard deviation.

64 ± 3 years. Whey protein was compared with a carbohydrate control (n = 12 comparisons), bovine colostrum (n = 1 comparison), skim milk powder (n = 1 comparison), and casein (n = 1 comparison). Whey protein supplementation dosage ranged from at least 6 g protein/day[16] to 48 g protein/day.[19] Daily total protein intakes were directly available from 6 studies (n = 8 comparisons) and were calculated from 3 additional studies. Total protein intake averaged 1.25 ± 0.19 g/kg/day in WP groups and 0.93 ± 0.17 g/kg/day in control groups ($P < 0.001$). Protein intake from dietary sources was 0.81 ± 0.17 g/kg/day among WP groups and 0.93 ± 0.17 g/kg/day among control groups ($P = 0.125$). Individual study details regarding contributions of dietary and supplemental protein to total protein intake (in grams per kilogram per day and percentage of total protein intake) are summarized in Table 4.[15–27] Fourteen of the 15 comparisons were assessed with body composition measured using dual-energy X-ray absorptiometry, whereas 1 used air displacement plethysmography.[22]

Quality of selected studies

Of the 13 articles included in this review, 3 articles[17,20,23] were deemed at low risk and 10 articles[15,16,18,19,21,22,24–27] had unclear risk of selection and detection biases based on provision of specific information pertaining to randomization and allocation concealment and specific methods for the blinding of the outcome assessment, respectively (Table 2). Seven articles[15,17,20,22,23,25,27] had low risk and the remaining 6 articles[16,18,19,21,24,26] had unclear risk for performance bias based on delineation of specific methods of participant and investigator blinding. No article was at high risk for bias in any domain. All 13 articles indicated that research staff provided supplements to the participants. Methods of measurement and assurance of compliance for the studies were described in 11 of the 13 articles.

Primary meta-analysis

Overall (15 comparisons), WP supplementation favored positive lean mass changes (WMD, 0.37 kg; 95%CI, 0.06–0.67) relative to a non-WP control (Figure 2). Whey protein supplementation did not influence changes in fat mass (Figure 3[15,16,18–27]) (WMD, −0.20 kg; 95%CI, −0.67 to 0.27) or body mass (WMD, −0.12 kg; 95%CI, −0.90 to 0.65), relative to a non-WP control (see Figure S1 in the Supporting Information online). Similar results occurred when WP was compared with carbohydrate controls (n = 10) (see Figure S2 in Supporting Information online). Whey protein supplementation favored positive lean mass changes (WMD, 0.36 kg; 95%CI, 0.01–0.70) but did not influence changes in fat mass (WMD, −0.22 kg; 95%CI, −0.75 to 0.31) or body mass (WMD, −0.03 kg; 95%CI, −0.84 to 0.78).

A priori subgroup analyses

Among studies without ER and with RT (n = 5 articles, n = 6 comparisons), WP did not influences changes in any body composition variable (see Figure S3 in

Table 3 **Descriptions of the randomized controlled trials included in a meta-analysis on the effects of whey protein supplementation versus a control on body composition changes in adult women**

	Study details			WP group details				Contrast group details			
References	Duration, wk	ER (+/−)	RT (+/−)	Type of supplement	No.	Age, y, mean ± SD	Protein supplemented, g/d	Type of supplement	No.	Age, y, mean ± SD	Protein supplemented, g/d
Adechian et al. (2012)[24]	6	+	−	MSP (WP)	17	32 ± 6	—	Casein	15	34 ± 4	—
Duff et al. (2014)[25]	8	−	+	WP	13	58 ± 6	38	Bovine colostrum	12	62 ± 5	38
Gordon et al. (2008)[18]	20	+	−	WP	9	57 ± 6	32	Dietary compensation	15	59 ± 7	0
Holm et al. (2008)[27]	8	−	+	WP + CHO + calcium	13	55 ± 4	10	CHO + calcium	16	55 ± 4	0
Keogh and Clifton (2008)[21]	52	+	−	WP (GMP enriched)	11	50 ± 12	30	Skim milk powder	9	50 ± 12	30
Kjølbæk et al. (2017)[20]	24	−	−	WP	32	42 ± 11	45	Maltodextrin	32	38 ± 11	0
Kjølbæk et al. (2017)[20]	24	−	−	WP + 1000 mg calcium	31	41 ± 11	45	Maltodextrin	32	38 ± 11	0
Martens et al. (2015)[22]	12	−	−	WP with α-lactalbumin	8	24 ± 5	—	Maltodextrin	10	24 ± 5	0
Mojtahedi et al. (2011)[17]	26	+	−	WP	13	65 ± 4	45	Maltodextrin	13	65 ± 5	0
Stragier et al. (2016)[26]	24	−	+	WP hydrolysate + 0.6 g LEU	7	64 ± 3	40	Maltodextrin	7	64 ± 3	0
Sukumar et al. (2011)[16]	52	+	−	WP	26	59 ± 4	—	Dietary compensation	21	57 ± 5	0
Taylor et al. (2016)[19]	8	−	+	WP	8	20 ± 2	48	Maltodextrin	8	21 ± 3	0
Verreijen et al. (2015)[23]	13	+	+	WP + LEU + vitamin D	16	64 ± 6	21	CHO	16	63 ± 6	0
Weinheimer et al. (2012)[15]	36	−	+	WP	41	47 ± 8	20	Maltodextrin	40	50 ± 6	0
Weinheimer et al. (2012)[15]	36	−	+	WP	29	50 ± 8	40	Maltodextrin	40	50 ± 6	0

Abbreviations: CHO, carbohydrate; ER, energy restriction; GMP, glycomacropeptide; LEU, leucine; MSP, milk soluble protein; RT, resistance training; WP, whey protein.

Table 4 **Descriptions of the contribution of dietary and supplemented protein towards total protein intake in a meta-analysis on the effects of whey protein supplementation versus a control on body composition changes in adult women**

	WP group details			Contrast group details		
References	Total protein intake, g/kg/d, mean ± SD[a]	Dietary protein, g/kg/d (% contribution)	Supplemented protein, g/kg/d (% contribution)	Total protein intake, g/kg/d, mean ± SD[a]	Dietary protein, g/kg/d (% contribution)	Supplemented protein, g/kg/d (% contribution)
Adechian et al. (2012)[24]	—	—	—	—	—	—
Duff et al. (2014)[25]	—	—	—	—	—	—
Gordon et al. (2008)[18]	1.3 ± 0.2	0.93 (71.5)	0.37 (28.5)	0.6 ± 0.1	0.6 (100)	0.00 (0)
Holm et al. (2008)[27]	1.10	0.94 (85.5)	0.16 (14.5)	0.94	0.94 (100)	0.00 (0)
Keogh and Clifton (2008)[21]	—	—	—	—	—	—
Kjølbæk et al. (2017)[20]	1.41 ± 0.48	0.86 (61.0)	0.55 (39.0)	1.17 ± 0.32	1.17 (100)	0.00 (0)
Kjølbæk et al. (2017)[20]	1.25 ± 0.44	0.72 (57.6)	0.53 (42.4)	1.17 ± 0.32	1.17 (100)	0.00 (0)
Martens et al. (2015)[22]	—	—	—	—	—	—
Mojtahedi et al. (2011)[17]	1.20 ± 0.14	0.63 (52.5)	0.57 (47.5)	0.86 ± 0.20	0.86 (100)	0.00 (0)
Stragier et al. (2016)[26]	1.23 ± 0.34	0.55 (44.7)	0.68 (55.3)	1.08 ± 0.30	1.08 (100)	0.00 (0)
Sukumar et al. (2011)[16]	1.04	—	—	0.78	0.78 (100)	0.00 (0)
Taylor et al. (2016)[19]	1.37	0.65 (47.4)	0.72 (52.6)	1.12	1.12 (100)	0.00 (0)
Verreijen et al. (2015)[23]	1.11 ± 0.28	0.87 (78.4)	0.24 (21.6)	0.85 ± 0.24	0.85 (100)	0.00 (0)
Weinheimer et al. (2012)[15]	1.13 ± 0.23	0.89 (78.8)	0.24 (21.2)	0.96 ± 0.28	0.96 (100)	0.00 (0)
Weinheimer et al. (2012)[15]	1.64 ± 0.38	1.01 (61.6)	0.63 (38.4)	0.96 ± 0.28	0.96 (100)	0.00 (0)

Abbreviation: WP, whey protein.
[a]Total protein intake is equal to dietary protein + supplemented protein.

References	Whey Protein Mean (kg)	Whey Protein SD (kg)	Whey Protein Participants (no.)	Control Mean (kg)	Control SD (kg)	Control Participants (no.)	Weight (%)	Mean difference (kg), random-effects (95% CI)
Adechian et al (2012)[24]	-3.3	1.3	17	-3.4	1.9	15	7.3	0.10 (-1.04 to 1.24)
Duff et al (2014)[25]	-0.2	0.89	13	0.1	1.42	12	8.4	-0.30 (-1.24 to 0.64)
Gordon et al (2008)[18]	-6.3	3	9	-6.3	3	15	2.8	0.00 (-2.48 to 2.48)
Holm et al (2008)[27]	-0.5	1.9	13	-0.6	2.3	16	5.4	0.10 (-1.43 to 1.63)
Keogh and Clifton (2008)[21]	-7.8	6.5	11	-7.3	6.1	9	0.7	-0.50 (-6.04 to 5.04)
Kjølbæk et al (2017)[20] -Ca	-0.2	4.6	31	0.2	3.2	38	4.1	-0.40 (-2.31 to 1.51)
Kjølbæk et al (2017)[20]	-0.1	4.3	32	0.2	3.2	38	4.4	-0.30 (-2.10 to 1.50)
Martens et al (2015)[22]	-0.3	0.5	8	0.3	0.6	10	11.2	-0.60 (-1.09 to -0.11)
Stragier et al (2016)[26]	-0.8	1.66	7	1.37	1.3	7	5.3	-2.13 (-3.70 to -0.57)
Sukumar et al (2011)[16]	-4.2	1.6	26	-4.5	0.9	21	9.7	0.30 (-0.43 to 1.03)
Taylor et al (2016)[19]	-1.3	1.3	8	-0.7	1.4	8	6.3	-0.60 (-1.92 to 0.72)
Verreijen et al (2015)[23]	-3	0.79	16	-1.8	1.11	16	10.1	-1.20 (-1.87 to -0.53)
Weinheimer et al (2012)[15]-20g	-0.2	0.53	41	-0.95	0.53	40	12.3	0.77 (0.54 to 1.00)
Weinheimer et al (2012)[15]-40g	-0.4	0.75	29	-0.95	0.53	40	12.0	0.52 (0.20 to 0.84)
Total							100	-0.20 (-0.67 to 0.27)

Heterogeneity: $Tau^2 = 0.452$; $Chi^2 = 64.17$, df = 13 (P = 0.000); $I^2 = 80\%$

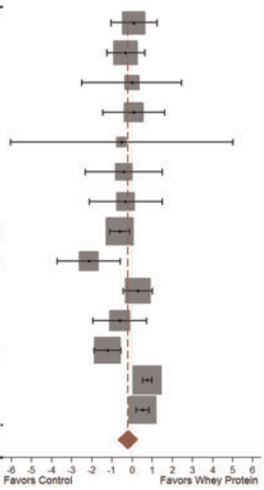

Figure 3 **Effect of whey protein supplementation on changes in fat mass in women.** A random-effects model was used for fat mass because heterogeneity was observed in pooled data. *Abbreviations*: Ca, calcium; CI, confidence interval; SD, standard deviation.

Supporting Information online). Lean mass (WMD, 0.22 kg; 95%CI, −0.19 to 0.64), fat mass (WMD, 0.08 kg; 95%CI, −0.46 to 0.62), and body mass (WMD, −0.23 kg; 95%CI, −1.41 to 0.96) changes were not different between groups.

Among studies without ER and RT (n = 2 articles, n = 3 comparisons), WP resulted in decreased fat mass (WMD, −0.57 kg; 95%CI, −1.03 to −0.11) without influencing changes in lean mass (WMD, 0.21 kg; 95%CI, −0.24 to 0.65) or body mass (WMD, −0.12 kg; 95%CI, −1.23 to 0.99).

Only 1 study (n = 1 article, n = 1 comparison) included both ER and RT; thus there were not enough data to conduct a meta-analysis for this condition.

Of all analyses (primary and subgroup), the most robust positive change in lean mass as a result of WP supplementation (WMD, 1.04 kg; 95%CI, 0.38–1.70) (Figure S4 in Supporting Information online) was found among studies with ER and without RT (n = 5 articles, n = 5 comparisons). Whey protein did not influence changes in fat mass (WMD, 0.22 kg; 95%CI, −0.37 to 0.81) or body mass (WMD, 0.48 kg; 95%CI, −0.51 to 1.47) (Figure S4 in Supporting Information online).

Secondary subgroup analyses

The beneficial effect of WP on lean mass was lost when only studies with RT were included in the analysis (n = 6 articles, n = 7 comparisons; WMD, 0.23 kg; 95%CI, −0.17 to 0.63) (Figure 4).[15–27] Results did not differ from the primary analysis when only studies without RT were included (n = 7 articles, n = 8 comparisons; WMD, 0.47 kg; 95%CI, 0.10 to 0.84) (Figure 4). The beneficial effect of WP on lean mass was more robust when only studies with an ER component were included (n = 6 articles, n = 6 comparisons; WMD, 0.90 kg; 95%CI, 0.31–1.49) (Figure 5).[15–27] There was no effect of WP on lean mass in studies without ER (n = 7 articles, n = 9 comparisons; WMD, 0.22 kg; 95%CI, −0.12 to 0.57) (Figure 5).

Sensitivity analyses

One-by-one removal of 14 of the 15 comparisons did not significantly influence the results or the statistical model used. Removal of the 20-g WP group from Weinheimer et al.[15] and removal of both Weinheimer et al. comparisons influenced the effect of WP on lean and fat mass. Specifically, the effect of WP on lean mass was either blunted (20-g WP group removed: WMD, 0.14 kg; 95%CI, 0.04–0.24) or ablated (both WP groups removed: WMD, 0.19 kg; 95%CI, −0.07 to 0.44), whereas the effect of WP on fat mass was strengthened (both WP groups removed: WMD, −0.48 kg; 95%CI, −0.86 to −0.10). Furthermore, removal of the 20-g group, or both the 20-g and >40-g groups permitted use of a fixed-effects model for lean mass changes, indicating reduced heterogeneity.

DISCUSSION

The goal of this systematic review and meta-analysis was to assess the effect of WP supplementation on body composition changes over time in adult women. Overall findings presented herein suggest that WP

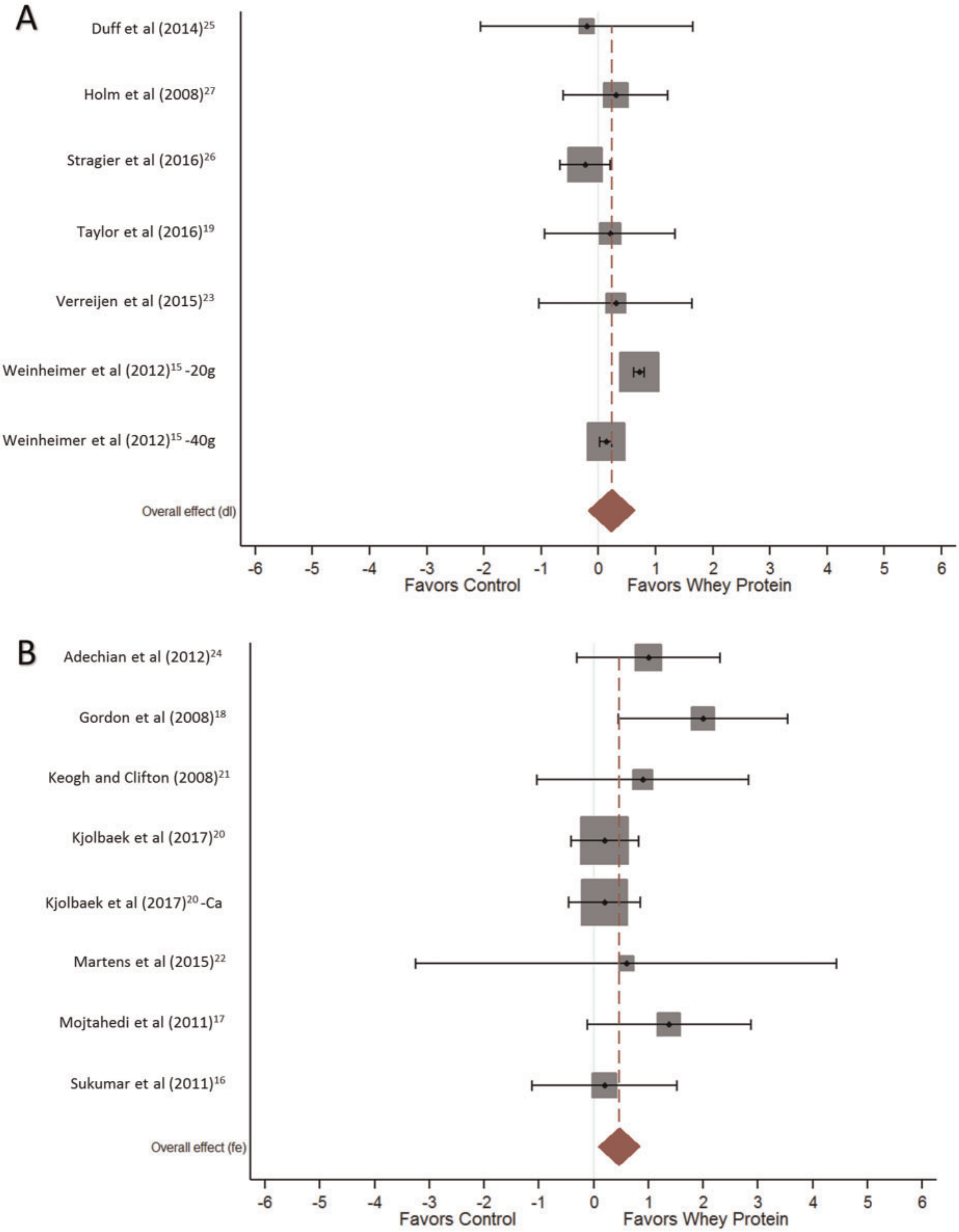

Figure 4 **Effect of whey protein supplementation on lean mass changes in women with or without resistance training.** *A*, Results of a random-effects meta-analysis representing pooled mean differences with 95% confidence intervals on lean mass in women participating in a resistance training protocol (WMD, 0.23 kg; 95%CI, −0.17 to 0.63). *B*, Results of a fixed-effects meta-analysis representing pooled mean differences with 95%CIs on lean mass in women not participating in resistance training (WMD, 0.47 kg; 95%CI, 0.10 to 0.84). *Abbreviations*: Ca, calcium; CI, confidence interval; WMD, weighted mean difference.

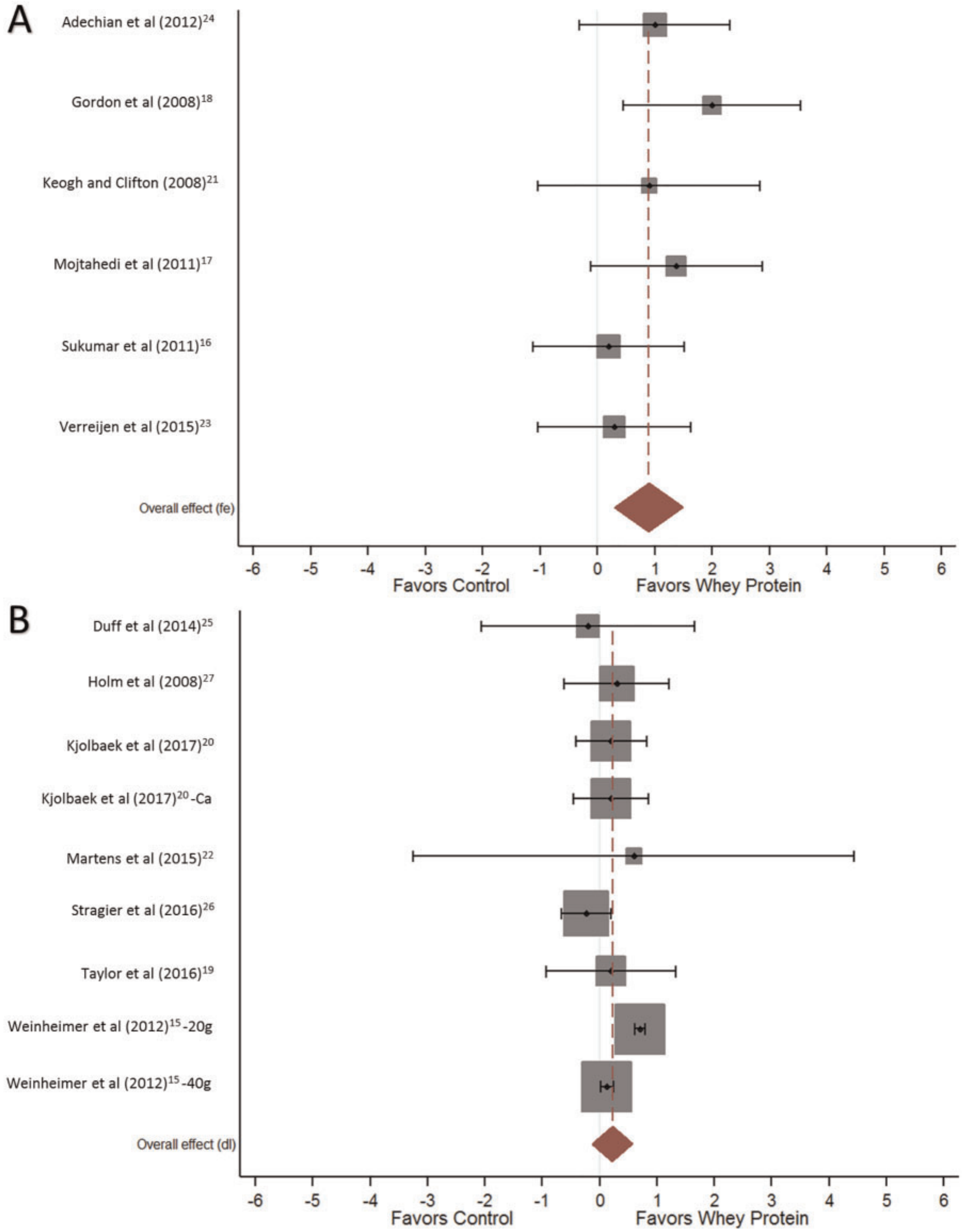

Figure 5 **Effect of whey protein supplementation on lean mass changes in women with or without energy restriction.** *A*, Results of a fixed-effects meta-analysis representing pooled mean differences with 95% confidence intervals on lean mass in women participating in studies with an energy restriction component (WMD, 0.90 kg; 95%CI, 0.31–1.49). *B*, Results of a random-effects meta-analysis representing pooled mean differences with 95%CIs on lean mass in women participating in studies without an energy restriction component (WMD, 0.22 kg; 95%CI, −0.12 to 0.57). *Abbreviations*: Ca, calcium; CI, confidence interval; WMD, weighted mean difference.

supplementation favors modest increases in lean mass, while not influencing fat mass or total body mass, irrespective of the state of energy sufficiency and exercise training. This moderate increase in lean mass over time (0.37 kg) represents <1% of the total lean mass of study participants and therefore does not support the public perception that WP causes excessive hypertrophy or "bulkiness" in adult women.

Some systematic reviews and meta-analyses of literature suggest that protein supplementation augments gains in lean mass,[3,10–12] whereas others indicate a null effect.[13,30] Potential discordance in findings could stem from reviews differing in inclusion criteria for age, training status, energy balance, and protein source. Despite these differences, one constant among all of these reviews is the inclusion of both sexes in analyses. There is a paucity of protein supplementation research in women, as discussed previously.[11] In line with this, women are underrepresented in protein supplementation meta-analyses; 68% of studies in the most-cited protein supplementation meta-analyses included only males.[12] Therefore, recommendations on the effectiveness of WP supplementation in women are of limited value. To address this concern, the present study was inclusive of only female participants in all of the analyses. Overall, these women-specific data are in agreement with the majority of meta-analyses inclusive of both sexes that find a modest increase in lean mass as a result of WP supplementation.

Another constant throughout previous systematic reviews and meta-analyses of protein supplementation literature is the inclusion of RT in featured studies.[3,10–13,30] In contrast, the present study features analyses with and without studies including RT in order to determine the separate and combined effects of WP supplementation and RT on body composition. One meta-analysis reported no overall effect of WP on lean mass in men and women, but subgroup analysis of only studies that included a RT component suggested WP supplementation increased lean mass.[30] In contrast with these findings, WP did not augment gains in lean mass in studies of women who performed RT (n = 6) but did result in increased lean mass relative to control in studies without RT (n = 7). Resistance training may be a potent enough anabolic stimulus that it washes out any potential effect of dietary protein manipulation on changes in lean mass.[31,32] Therefore, the secondary subgroup analyses suggest that the beneficial effect of WP on lean mass in women is more robust in the absence of RT.

Pooled data on the effects of protein supplementation on fat mass and body mass are limited, compared with assessments of lean mass. One meta-analysis reported no effect of protein supplementation on fat mass,[12] whereas another suggested protein supplementation reduces fat mass (without influencing body mass).[30] The only meta-analysis with data on fat mass and body mass specific to WP concluded that WP supplementation significantly reduced fat mass and body mass.[30] The present findings in a female-only population are inconsistent with these results because there was no detected effect of WP supplementation on changes in either fat mass or body mass. Likely the most influential factor on changes in fat mass is energy restriction.[33] The lack of ER stimulus in over half of the comparisons (n = 9) in the primary analysis may have washed out or not permitted the potential fat mass–reducing effects of WP supplementation to manifest. However, fat mass and body mass findings from secondary subgroup analysis inclusive of only studies with an ER component did not differ from the primary analysis. Presence or absence of ER most strongly influenced differential changes in lean mass from WP or control supplementation. There was no effect of WP on lean mass in analysis of studies without ER, whereas there was a pronounced positive difference in lean mass between WP and control in studies with ER. These findings are in line with the sentiment that higher protein intake may be of greater importance for promoting positive changes in body composition during weight loss, relative to potential benefits of higher protein intake during weight maintenance.[34]

A priori subgroup analyses more precisely assessed the separate or combined potential for ER and RT to modulate the effects of WP supplementation on body composition. There were not enough studies to permit meta-analysis on the effects of WP on body composition in groups who were participating in both ER and RT (n = 1). In comparisons with RT but without ER (n = 6), WP supplementation did not influence changes in lean mass, fat mass, or body mass. Likewise, in comparisons without both ER and RT (n = 3), there was no effect of WP on any body composition outcome. In secondary subgroup analyses, the effects of WP on lean mass are amplified in studies with ER and blunted in studies with RT. This claim is further supported by subgroup analysis of studies featuring ER without RT (n = 5), which presented the most robust effects of WP supplementation on lean mass of all analyses (1.04 kg vs 0.37 kg in overall analysis). Therefore, these findings suggest WP supplementation may be less effective when energy needs are met and more effective in conditions where increased dietary protein is purported to be of increased importance (ER).

Strengths and limitations

This review is subject to standard limitations of systematic reviews and meta-analyses such as publication bias and inconsistencies in experimental features of selected studies. To address this limitation, manual searches of relevant systematic reviews and meta-analyses were conducted. The original goal was to determine the effects of WP supplementation on body composition in women in 4 conditions in a 2×2 factorial manner (with and without RT, with and without ER). Due to a paucity of data in specific subgroups, firm conclusions about the effects of WP on body composition with respect to specific energy and training statuses cannot be reached. Another consideration is that total protein intake was greater in the WP groups when compared withe control groups. Therefore, differential changes in body composition may be attributable to greater total protein intake[35] as opposed to specifically WP supplementation.[15] However, specific effects of WP on body composition cannot be disentangled from the overall effects of greater daily total protein intake (seen in WP groups) on body composition because only 3 comparisons included in this meta-analysis assessed WP versus another protein source; 12 comparisons were between WP and a carbohydrate control.

Only 5 of the studies included in this review were in female-only populations.[16–19,27] Because body composition data in the remaining studies were typically presented in manuscripts with means and SDs for mixed-sex groups, acquisition of data was challenging. Therefore, more studies conducted with female participants as part of a mixed-sex populations exist than are reported herein. Future studies should include sex-specific data in manuscripts or supplemental tables. Additionally, a random-effects model was used in most analyses due to inherent heterogeneity in studies assessed. To address this shortcoming, sensitivity analyses were conducted to determine potential sources of heterogeneity. Removal of any single comparison did not significantly influence findings on body composition outcomes, including removal of 1 comparison,[15] which permitted use of a fixed-effects model.

CONCLUSION

In summary, findings from this systematic review and meta-analysis indicate that WP supplementation improves body composition in adult women by modestly increasing lean mass without influencing changes in fat mass. This null effect on fat mass and <1% increase in lean mass is not in line with the public perception that WP causes excessive hypertrophy or "bulkiness" in adult women. Whey protein may be more beneficial for improving body composition when included as part of a weight loss program. Although more research is needed to specifically assess the effects in varying states of energy sufficiency and exercise training, the overall findings support consumption of WP in women seeking to modestly improve body composition.

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Author contributions. R.E.B., J.L.H., and W.W.C. designed the research; R.E.B. and J.L.H conducted the research; R.E.B. analyzed the data; R.E.B, J.L.H., and W.W.C. wrote the manuscript and had primary responsibility for the final content of the manuscript. All authors read and approved the final manuscript.

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Declaration of interest. R.E.B. and J.L.H have no conflicts of interests to declare. W.W.C received research funds from the National Pork Board, American Egg Board-Egg Nutrition Center, National Dairy Council, and National Cattlemen's Beef Association and served on the National Dairy Council's Whey Protein Advisory Panel during the time this study was being conducted.

Supporting Information

The following Supporting Information is available through the online version of this article at the publisher's website.

Table S1 **Search strategy**

Figure S1 **Effect of whey protein supplementation on changes in body mass in women**

Figure S2 **Effect of whey protein supplementation versus carbohydrate control on changes in lean mass, fat mass, and body mass in women**

Figure S3 **Effect of whey protein supplementation on changes in lean mass, fat mass, and body mass in women without energy restriction and with resistance training**

Figure S4 **Effect of whey protein supplementation on changes in lean mass, fat mass, and body mass in women with energy restriction and without resistance training**

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