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EFFECTS OF MILK PROTEINS SUPPLEMENTATION IN OLDER ADULTS UNDERGOING RESISTANCE TRAINING: A META-ANALYSIS OF RANDOMIZED CONTROL TRIALS

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Abstract: *Background:* Older adults experience age-related physiological changes that affect body weight and body composition. In general, nutrition and exercise have been identified as potent stimulators of protein synthesis in skeletal muscle. Milk proteins are excellent sources of all the essential amino acids and may represent an ideal protein source to promote muscle anabolism in older adults undergoing resistance training. However, several randomized control trials (RCTs) have yielded mixed results on the effects of milk proteins supplementation in combination with resistance training on body weight and composition. *Methods:* PubMed, Web of Science and Cochrane databases were searched for literature that evaluated the effects of milk proteins supplementation on body weight and composition among older adults (age ≥ 60 years) undergoing resistance training up to September 2016. A random-effects model was used to calculate the pooled estimates and 95% confidence intervals (CIs) of effect sizes. *Results:* The final analysis included 10 RCTs involving 574 participants (mean age range from 60 to 80.8 years). Overall, the combination of milk proteins supplementation and resistance training did not have significant effect on fat mass (0.30, 95% CI -0.25, 0.86 kg) or body weight (1.02, 95% CI: -0.01, 2.04 kg). However, a positive effect of milk proteins supplementation paired with resistance training on fat-free mass was observed (0.74, 95% CI 0.30, 1.17 kg). Greater fat-free mass gains were observed in studies that included more than 55 participants (0.73, 95% CI 0.30, 1.16 kg), and in studies that enrolled participants with aging-related medical conditions (1.60, 95% CI 0.92, 2.28 kg). There was no statistical evidence of publication bias among the studies. *Conclusion:* Our findings provide evidence that supplementation of milk protein, in combination with resistance training, is effective to elicit fat-free mass gain in older adults.

Key words: Body weight, fat mass, fat-free mass, milk proteins, resistance training.

Introduction

The global rapid growth of aging population has become a public health issue. The World Health Organization has estimated that the expansion of the global population over 60 years of age will increase from 605 million to greater than two billion between 2000 and 2050 (1). Older adults experience age-related physiological changes that affect body composition, including body weight gain, increased fat mass and decreased fat-free mass (2). Progressive loss of skeletal muscle mass or sarcopenia is associated with increased susceptibility of injury and disability, increased health risk, and reduced quality of life in older adults (3, 4). Although the exact mechanisms underlying sarcopenia remain to be investigated, age-related impairment in ability for skeletal muscle of older adults to respond to anabolic stimuli is believed to be one of the primary factors that contribute to muscle loss in the elderly (5). In general, nutrition and exercise have been identified as potent stimulators of protein synthesis in skeletal muscle (6-9). In that regard, milk proteins are excellent sources of all the essential amino acids, are readily available commercially, and are relatively inexpensive (10); thus, it may represent an ideal protein source to promote muscle anabolism and protein synthesis in older adults undergoing resistance training. However, several randomized control trials (RCTs) have yielded mixed results on the effects of milk protein supplementation in combination with resistance training on body weight and composition (11-14). Furthermore, recent meta-analyses by Cermak et al. (15) and Finger et al. (16) found that protein supplementation, when combined with regimented resistance training, enhanced fat-free mass in older adults. Although most intervention groups of trials in both meta-analyses were supplemented with milk proteins, the effects of milk proteins supplementation on body weight and composition (fat-free mass and fat mass) have not yet been specifically investigated. Rather, inclusion criteria encompassed studies in which at least one intervention group consumed a protein and/or amino acids supplement during resistance training. Therefore, we conducted a meta-analysis of randomized control trials (RCTs) to specifically examine the effect of milk proteins supplementation on body weight and body composition in older adults undergoing resistance training.

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Methods

Search Strategy

This meta-analysis was planned, conducted, and reported according to the preferred reporting items for systematic reviews and meta-analyses (PRISMA) recommendation (17). We searched PubMed, Web of Science and Cochrane databases for literature that evaluated the effects of milk proteins supplementation on body weight and composition among older adults undergoing resistance training up to September 2016. We used the following search algorithm: ('milk' OR 'whey' OR 'casein' OR 'protein supplementation') AND ('older adults' OR 'elderly' OR 'old age') AND ('body composition' OR 'body weight' OR 'body mass' OR 'body fat' OR 'fat mass' OR 'lean body mass' OR 'lean body tissue' OR 'fat-free mass' OR 'lean mass') AND ('resistance training' OR 'resistance exercise' OR 'training' OR 'exercise'). The search strategy had no language, publication date, or publication type restrictions. In addition, the reference lists of retrieved full publications and previous meta-analysis were reviewed to complement the search and to identify relevant studies that were missed during the electronic database search.

Eligibility Criteria

To be included in this meta-analysis, the studies had to meet the following inclusion criteria: (a) RCTs lasted at least 12 weeks; (b) one or more intervention groups receiving milk proteins supplementation (e.g. whey protein, casein) or dairy products (e.g. milk); (c) All participants were involved in resistance training; (d) The control groups could either be assigned to resistance training alone (without supplementation) or resistance training with non-protein supplementation (e.g. carbohydrate beverage); (e) participants with a mean age of ≥60 years; (f) Fat-free mass, fat mass, or body weight was evaluated. In the case of multiple publications with overlapping data from the same trial, the article with the most detailed information was selected.

Data extraction and Quality Assessment

Using a standardized data-collection form, the following study characteristics were abstracted from each study: first author's last name, publication year and country of origin, the details of population characteristics (mean age, age range, sex distributions and health status of subjects), study duration, training details (frequency, type, and volume), supplementation details (amount, type, timing, and frequency), and outcomes assessed. The risks of selection, performance, and detection biases were evaluated from selected studies using a modified Cochrane tool for assessing risk of bias (18) (Table S1).Two investigators (K. H. and C.-G. C.) independently performed the literature search, data extraction, and quality assessment. Any discrepancies regarding inclusion were resolved by consensus. Because there was complete agreement between both reviewers regarding data searching and extraction and quality assessment, a kappa statistic was not calculated.

Statistical Analysis

Milk proteins supplementation together with resistance training was considered as the intervention arm in this meta-analysis. Resistance training with or without placebo was considered as control arm. The net changes of each outcome in the intervention and control groups were reported as differences between mean values at baseline and endpoint. For studies reporting only the mean difference between the intervention and control groups, we set the mean change of control group as zero and the mean change of intervention group as the reported mean difference. Studies with no reported Standard deviation (SD) values, had their values imputed using a standard formula (18). If only SD for the baseline and final values were provided, we computed SD for net changes using the method proposed by Folmann et al. (19) in which a correlation coefficient of 0.5 was assumed.

The degree of heterogeneity across trials was assessed using Q and I^2 statistics. For the Q statistic, $P<0.1$ was considered statistically significant; and for the I^2 statistic, the following conventional cut-off points were used: <25% (low heterogeneity), 25-50% (moderate heterogeneity), and >75% (severe heterogeneity). Both Begg's rank correlation test and Egger's linear regression test were performed to investigate potential publication bias (20). If evidence of publication bias was observed, the trim and fill method was applied to correct the bias (21). A random-effects model was used to calculate the pooled estimates and 95% CIs of effect sizes. To explore the possible influences of study and participant characteristics on combined effect sizes, subgroup and meta-regression analyses were performed according to sex distributions, mean age of subjects, trial duration, number of subjects, health status of subjects, protein type, amount of extra protein, and supplementation frequency. In addition, we conducted sensitivity analysis to investigate the influence of a single trial on the overall effect estimated by omitting one trial in each turn. All analyses were performed using STATA version 11.0 (StataCorp, College Station, TX, USA). A P value < 0.05 was considered to be statistically significant, unless otherwise specified.

Results

Study Characteristics

We identified 10 studies (11-14, 22-27) that fully met our inclusion criteria for this meta-analysis. A flow chart of study selection, including reasons for exclusion, is presented in Figure 1. The characteristics of the included studies are presented in Table 1.The included studies were published between 1995 and 2016. The sample size of individual trial varied from 12 to 130 participants. The mean age of the 574 participants ranged from 60 to 80.8 years. Although crossover design was not our exclusion criteria, all included studies had parallel design.

Table 1
Characteristics of the included studies

First author, year	Country	Sex	Mean age (number of sub-jects)	Health Status of subjects	Training details				Supplementation details					Outcome assessed BW, FM, FFM
					Frequency and length	Type	Exercise number	Training volume	Protein type Milk-based beverages	Amount of extra protein 0.8 g/kg	Placebo Low-protein diet	Protein timing NRE	Frequency ED	
Campbell, 1995 (11)	United States	M/W	I: 64 (6); C: 66 (6)	Healthy	3 d/w × 12w	UB + LB	4	3 sets × 8-12 reps or fatigue						
Candow, 2006 (12)	Canada	M	I1: 63.3 (9); I2: 66.5 (10); C: 64.6 (10)	Healthy	3 d/w × 12 w	UB + LB	9	3 sets×10 reps	Whey protein, casein and egg albumin	0.3 g/kg	Flavored carbohy-drate beverage	B, A	TD	FFM
Eliot, 2008 (22)	United States	M	48-72 (42)	Healthy	3 d/w ×14 w	UB + LB	8	3 sets × 8 reps	Whey protein concentrate	35 g/d	Carbohydrate beverage	A	TD	BW, FM, FFM
Verdijk, 2009 (23)	Netherlands	M	I: 72 (13); C: 72 (13)	Healthy	3 d/w×12 w	LB	2	4 sets × 8-15 reps	Casein hydrolysate	20 g/d	Flavored beverage	B, A	TD	BW, FFM
Kukuljan, 2009 (24)	Australia	M	I: 61.7 (45); C: 59.9 (44)	Healthy	3 d/w ×72 w	UB + LB	6a	2-3 sets ×8-20 reps	Fortified milk (Calcium and Vitamin D3)	13.2 g/d	No supplemen-tation	NRE	ED	BW, FM, FFM
Tieland, 2012 (13)	Netherlands	M/W	I: 78 (31); C: 79 (31)	Frail	2 d/w ×24 w	UB + LB	6	3 (UB)-4 (LB) ×8-15 reps	Milk protein concentrate	15 g/d	Flavored carbohy-drate beverage	NRE	ED	BW, FM, FFM
Chalé, 2013 (25)	United States	M/W	I: 78 (42); C: 77.3 (38)	Mobility-limited	3 d/w×12 w	UB + LB	5	2-3 sets × 10-12 reps	Whey protein concentrate	40 g/day	Isocaloric malto-drexin	A	ED	BW, FM, FFM
Leenders, 2013 (26)	Netherlands	M/W	I1: 72 (12); I2: 70 (15); C1: 69 (12); C2: 70 (14)	Healthy	3 d/w ×24 w	UB + LB	9	3-4 sets × 8-15 reps	Milk protein concentrate	15 g/day	Fat and protein free beverage	NRE	ED	FFM
Verreijen, 2015 (27)	Netherlands	M/W	I: 63.7 (30); C: 63 (30)	Obese	3 d/w ×13 w	UB + LB	10	3 sets ×12-20 reps	Whey protein	20 g/day	Isocaloric product	A	ED	BW, FM
Rondanelli, 2016 (14)	Italy	M/W	I: 80.8 (69); C: 80.2 (61)	Sarcopenic	5 d/w ×12 w	UB + LB	4b	≤ 8 reps	Whey protein	22 g/day	Isocaloric malto-drexin	NRE	ED	BW, FM, FFM

A after exercise; B, A before and after exercise; BW body weight; C control; ED every day; FM fat mass, I intervention subjects; I ½ intervention subjects groups 1 and 2; LB lower-body exercises; FFM free-fat mass; M men; NRE not related to exercise; reps repetitions; TD training day; UB upper-body exercises; w week(s); W women. a. Refers to the number of exercises comprised in seated-chair training program; b. Refers to the number of exercises comprised in resistance band training program

Table 2
Subgroup analyses of mean change in fat-free mass, fat mass and body weight

Subgroup	Change in fat-free mass			Change in fat mass			Change in body weight		
	No.	Effect (95% CI)	P	No.	Effect (95% CI)	P	No.	Effect (95% CI)	P
Total	9	0.74 (0.30, 1.17)		7	0.30 (-0.25, 0.86)		8	1.02 (-0.01, 2.40)	
Sex									
Men/women	4	0.79 (0.09, 1.49)	0.869	4	-0.15 (-0.87, 0.56)	0.101	4	0.70 (-1.05, 2.46)	0.742
Men	5	0.68 (0.07, 1.29)		3	0.30 (-0.25, 0.86)		4	1.38 (-0.02, 2.77)	
Mean Age									
≤65	3	0.30 (-0.44, 1.06)	0.342	3	0.11 (-0.70, 0.92)	0.855	3	1.84 (0.73, 2.94)	0.507
>65	6	0.88 (0.28, 1.47)		4	0.21 (-1.00, 1.42)		5	0.61 (-0.94, 2.17)	
Study length									
12 weeks	5	0.60 (-0.04, 1.25)	0.613	3	0.03 (-0.79, 0.86)	0.641	3	1.26 (-0.23, 2.75)	0.808
>12 weeks	4	0.86 (0.23, 1.49)		4	0.34 (-0.79, 1.46)		5	0.77 (-0.92, 2.46)	
Number of subjects									
≤55	5	0.27 (-0.22, 0.76)	0.011	2	0.15 (-1.46, 1.76)	0.948	3	0 (-1.97, 1.96)	0.422
>55	4	0.73 (0.30, 1.16)		5	0.25 (-0.53, 1.02)		5	1.23 (-0.10, 2.55)	
Health status of subjects									
Relatively Healthy	6	0.32 (-0.16, 0.79)	0.014	3	0.03 (-1.42, 1.48)	0.571	4	0.13 (-1.68, 1.95)	0.445
With aging-related medical conditions	3	1.60 (0.92, 2.28)		3	0.57 (-0.28, 1.42)		3	1.23 (-0.25, 2.70)	
Protein types									
Whey	3	1.53 (0.64, 2.42)	0.128	4	-0.08 (-0.80, 0.64)	0.161	4	0.65 (-1.16, 2.46)	0.701
Other milk proteins	6	0.56 (0.12, 0.99)		3	0.84 (-0.04, 1.73)		4	1.43 (0.11, 2.75)	
Protein dose									
≤20 g/d	4	0.69 (0.03, 1.35)	0.273	3	0.22 (-1.28, 1.71)	0.885	4	0.65 (-1.11, 2.40)	0.600
>20 g/d	3	1.53 (0.64, 2.42)		3	0.14 (-0.70, 0.98)		3	1.82 (0.71, 2.94)	
Supplement frequency									
Everyday	6	1.04 (0.48, 1.59)	0.114	6	0.24 (-0.41, 0.89)	-	6	1.21 (0.02, 2.40)	0.423
Training day only	3	0.24 (-0.40, 0.88)		1	0.60 (-1.83, 3.03)		2	-0.15 (-2.34, 2.04)	

CI, confidence interval; P value for for heterogeneity according to meta-regression.

Dietary compliance was measured by return of supplement packets or daily food records. Several studies (13, 24, 25, 27) reported good compliance with the supplementations, while others had no judgement. Characteristics of participants enrolled in these trials varied across studies. Notable differences in population characteristics included mobility-limited elderly in one study (25), obese elderly in one study (27), frail elderly in one study (13) and sarcopenic elderly (14) in one study. Regarding the sex of the participants, four studies were conducted exclusively in men (12, 22-24) and the remaining six were in both sexes (11, 13, 14, 25-27). Four (13, 23, 26, 27) of the included studies were conducted in Netherlands, three (11, 22, 25) in the United States, one (24) in Australia, one (14) in Italy , and one (12) in Canada.

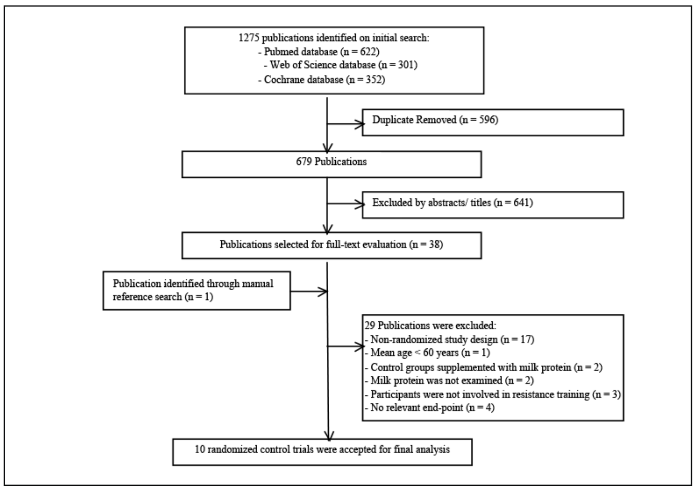
Milk Proteins Supplementation Characteristics

The amount, frequency, and timing of milk proteins and control supplementation varied largely across studies. Regarding the amount of protein, only two studies reported protein quantity according to body weight (0.3 and 0.8 g/kg body weight/day). The remaining eight studies reported protein quantity according to daily amounts, with amounts of extra protein ranging from 14.2 g/day to 40 g/day. The sources of protein supplementation were different in intervention group of each study, consisting of milk-based beverages, a combination of whey protein, casein and egg albumin, whey protein concentrate, casein hydrolysate, fortified milk, milk protein concentrate, and whey protein. Seven studies (11, 13, 14, 24-27) provided daily protein supplementation during trial, whereas in the remaining three studies (12, 22, 23) protein supplementation was consumed on training days only. Regarding the control groups, the placebo used varied between studies and included isocaloric products, flavored beverages, low protein diet, and carbohydrate beverages.

Resistance Training Characteristics

The type, frequency, volume, and intensity of resistance exercise varied substantially across studies. The duration of interventions lasted between 12 to 72 weeks. The mean of exercise frequency was three workout sessions per week (ranged from two days per week to five days per week). The mean of resistance training intensity was 79% of 1 repetition maximum (RM) (ranged from 70-85%). Among ten studies, nine studies (11-14, 22, 24-27) prescribed whole-body training regimens, and only one study (23) prescribed lower-body exercises. The number of different exercises completed per session ranged from 2 to 10 exercises. The number of sets per exercise during each workout ranged from 2 to 4 sets. The number of repetitions per set varied from 8 to 20 repetitions.

Figure 1
Flow chart of study selection of articles included in the present meta-analysis



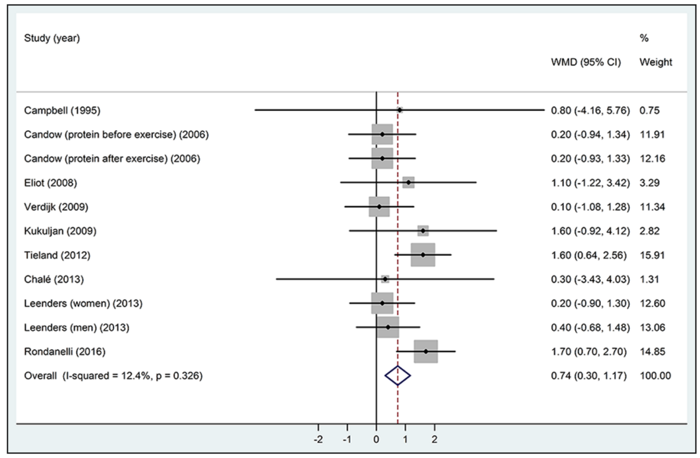
Quality of Included Studies

Among the included studies, 40 % presented adequate random sequence generation (four of 10), 40 % reported allocation concealment (four of 10), 70 % had blinded participants and study investigators (seven of 10) and 70 % blinded assessment of outcomes (seven of 10).

Effect of Milk Proteins Supplementation on Fat-Free Mass, Fat Mass and Body Weight

Compared with control groups undergoing resistance training only, milk proteins supplementation was associated with an average net change ranging from 0.1 to 1.7 kg for fat-free mass, from -0.7 to 1.2 kg for fat mass, and -0.6 to 2.3 kg for body weight. Increase in fat-free mass was observed in the groups receiving milk proteins (0.74, 95% CI 0.30, 1.17 kg; I^2 = 12.4%, P-heterogenity = 0.326) (Figure 2). However, there was no noteworthy effect of milk proteins supplementation on fat mass (0.30, 95% CI -0.25, 0.86 kg; I^2 = 0%, P-heterogenity = 0.43) (Figure 3) and body weight (1.02, 95% CI -0.01, 2.04 kg; I^2 = 35.3%, P-heterogenity = 0.147) (Figure 4). There was no statistical evidence of publication bias among the studies on fat-free mass (Begg, P = 0.81 and Egger, P = 0.89), fat mass (Begg, P = 1.00 and Egger, P = 0.40) and body weight (Begg, P = 0.62 and Egger, P = 0.26), respectively.

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 (weighted mean difference: 0.74 kg; 95% CI: 0.30-1.17, I^2 = 12.4%, P-heterogenity = 0.326)



Subgroup and Sensitivity Analyses

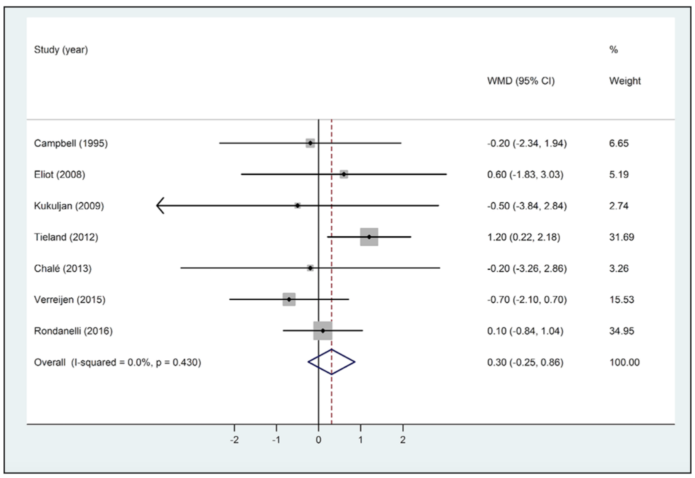
The results of the sub-group analyses stratified by sex distributions, mean age of subjects, trial duration, number of subjects, health status of subjects, protein type, amount of extra protein and supplementation frequency are presented in Table 2. Greater fat-free mass gains were observed in studies that included more than 55 participants (0.73, 95% CI 0.30, 1.16 kg), and in studies that enrolled participants with aging-related medical conditions (frail, mobility limited and sarcopenic) (1.60, 95% CI 0.92, 2.28 kg). There was no significant change in fat-mass and body weight across subgroups. Sensitivity analyses examining the impact of a single trial on the overall results by omitting one trial in each turn yielded a range from 0.55 kg (95% CI 0.14, 1.00) to 0.82 kg (95% CI 0.41, 1.24) for fat-free mass, from -0.11 kg (95% CI -0.78, 0.55) to 0.49 kg (95% CI -0.11, 1.09) for fat mass, and from 0.85 kg (95% CI -0.32, 1.74) to 1.71 kg (95% CI 0.92, 2.49) for bodyweight.

Discussion

The present meta-analysis is the first quantitative review of randomized control trials evaluating the effects of milk proteins supplementation on changes in body weight and body composition of older adults undergoing resistance training. Overall, the combination of milk proteins supplementation and resistance training did not have significant effect on fat mass and body weight. However, a positive effect of milk proteins supplementation paired with resistance training on fat-free mass was observed. These discrepancies are difficult and challenging to explain. Although speculative, the involvement of resistance training in these studies may have prevented changes in body weight and fat mass due to milk proteins supplementation.

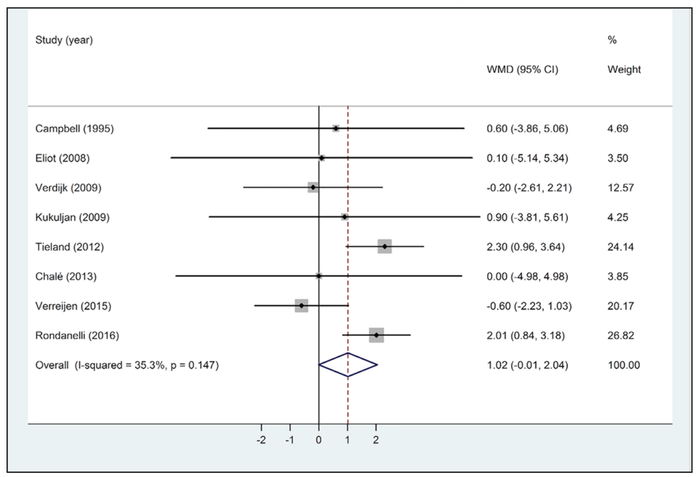
Most of milk proteins supplements contain around 100 calories per supplement; when milk proteins supplementation was taken three to five times per week, the participants received an additional 300 calories per week or higher. Therefore, it is expected that the additional caloric intake from milk proteins supplementation may lead to increased fat mass as well as weight gain. However, this potential body weight and fat mass gains from additional caloric intake was apparently attenuated by the increased in total energy expenditure from the resistance training, resulting in noteworthy gains in both body weight and fat mass.

Figure 3
Forest plot of the results of a random-effects meta-analysis shown as pooled mean differences with 95% CIs on fat mass (weighted mean difference: 0.30 kg; 95% CI: -0.25-0.86, I^2 = 0%, P-heterogenity = 0.430)



Meta-analyses by Cermak et al. (15) and Finger et al. (16) suggest that protein supplementation, when combined with regimented resistance training, enhanced fat-free mass in older adults. In this present meta-analysis, we only included studies which examined the effects of milk proteins on body weight and body composition in older adults undergoing resistance training instead of studies in which at least one intervention group consumed proteins from any sources. There are several reasons why we need to specifically examine the effect of milk proteins on body weight and body composition in older adults. First, milk and dairy products are widely consumed around the world on a daily basis. Second, milk proteins are excellent sources of all the essential amino acids, are readily available commercially, and are relatively inexpensive (10). Third, although consuming whole foods is the preferable way to obtain adequate protein, older adults may also have difficulty consuming adequate protein from diet alone due to several physiological factors that may influence food intake such as appetite, disturbances in taste and smell (28, 29), poor dental health (30), changes in digestive functions (31), dementia (32), and age-related impairments in the regulation of food intake (33). For example, eggs, lean meats, poultry, fish, and soy-based foods all contain high-quality protein and they are fairly inexpensive and easy to find. However, obtaining protein from whole foods can be a serious problem for older adults since it requires the ability to cook and chew, not to mention other physiological barriers experienced by older adults. Hence, it is reasonable to consider milk proteins supplements-which usually available in powder or liquid forms-as convenience way to meet protein needs may make sense. Finally, there is emerging evidence that the combination of milk proteins supplementation and resistance training may enhance fat-free mass in older adults (13, 14, 25). Thus, we hypothesize milk proteins may represent an ideal protein source to promote muscle anabolism and protein synthesis, resulting in greater gain in fat-free mass in older adults undergoing resistance training.

Figure 4
Forest plot of the results of a random-effects meta-analysis shown as pooled mean differences with 95% CIs on body weight (weighted mean difference: 1.02 kg; 95% CI: -0.01-2.04, I^2 = 35.3%, P-heterogenity = 0.147)



Our findings showed that studies using whey protein as dietary supplementation had greater fat-free mass gain, compared with studies using other kinds of milk proteins (i.e. casein, milk protein concentrated) as supplementation. We considered several possible explanations for this difference. First, whey protein is digested and absorbed rapidly, resulting in fast, high and transient elevation in plasma amino acid concentrations (hyperaminoacidemia). Hyperamidoacidemia stimulates an increase in muscle protein synthesis and anabolism (34, 35). Second, "fast-acting" protein might be more beneficial than"slow-acting" protein to limit protein loss in older people (36). Third, whey protein is rich in essential amino acid leucine, which plays a key role in muscle protein synthesis, and affects muscle hypertrophy (37, 38). Consumption of approximately 3 to 4 g of leucine is required to promote optimal protein synthesis (39, 40). Therefore, whey protein that provides at least 3 g of leucine per serving may serve as an ideal post-workout supplement in older adults. In addition, consuming repeated doses of whey protein may be beneficial to overcome the short-lived and transient nature of whey protein.

When analyzing subgroups according to health status of the participants, studies that included participants with aged-related medical conditions (frail, mobility limited and sarcopenic) achieved greater gain in fat-free mass than studies that included relatively healthy participants. Greater effect of milk proteins on fat-free mass during resistance training in participants with aged-related medical conditions is reasonable, since these individuals generally consume dietary protein below the average protein requirement, and tend to have lower fat-free mass than healthy participants (41). Moreover, genetic predisposition to poor appetite, physiological changes and medical conditions that lead to age- and disease-related anorexia, age-associated decline in physical and mental functioning that limits shopping and food preparation, and food insecurity due to financial and social constraints could be the reasons for older adults fail to meet protein requirement (42, 43).

Furthermore, participants who consumed higher doses of protein gain more fat-free mass than participants who consumed lower doses. Older adults may develop anabolic resistance, a phenomenon in which skeletal muscle fail to respond to stimulus with normal muscle protein synthesis, thus leading to slower tissue remodeling (44). The exact mechanism of anabolic resistance has not yet been determined, and several complex mechanisms have been hypothesized, including reduced protein digestion and amino acid absorption (45), reduced vasodilatory absorption of skeletal muscle to insulin (46), reduced dietary amino acid uptake in muscle (47), and impairment of mammalian target of rapamycin complex (mTORC)1 signaling (48). As the consequence of this phenomenon, older adults may require more protein/kilogram body weight than do younger adults. Thus, the observed positive effect on fat-free mass of those who consumed higher doses of protein is reasonable.

Finally, several issues warrant additional discussion. First, although older adults may indeed benefit from a higher protein intake, it should be highlighted that the optimal level of daily protein intake is not yet established and can vary greatly depending on a range of factors, including amount and type of physical activity, frequency and volume of training, etc. Second, any protein supplementation trials need to ensure an adequate energy intake to meet energy demands. In the absence of adequate energy the protein will be metabolized as an energy source in order to maintain vital organs and functions. Third, since certain types of protein happens to be digested slower or faster than any others, examining the optimal type of protein, timing of protein consumption, and distribution of protein may reveal why certain milk protein has greater effect than another on body weight and body composition outcomes. Altogether, the positive effect of milk proteins supplementation and resistance training on fat-free mass may be further extended to muscle mass through optimal dosage, type of protein, protein timing and frequency, and type and frequency of resistance exercise.

Our analysis did have limitations. First, differences in trial duration, subject characteristics (i.e. age, sex distributions, and health status), supplement protocol (i.e. dose, type of protein, timing, and frequency), and resistance training protocol (i.e. frequency, type, and volume) may all contribute to the variation in trial effects, making it harder to compare the results. Moreover, the format in which the data were presented in each studies varied widely, which made the data extraction difficult and may have influenced the extracted result. Therefore, results from our meta-analysis should always be interpreted with caution. Second, only one (11) of the ten studies included in our meta-analysis analyzed the timing of protein supplementation (before and after training) related to training session. A literature review by Stark et al. (49) put forward that the timing of protein supplementation was important, and consumption of milk protein immediately following resistance exercise was effective in promoting a positive net protein balance, which leads to increases in fat-free mass, and decreases in body fat. Third, the combination of milk protein, particularly whey protein with vitamin D (14), may also affect results. Although vitamin D supplementation is not known to augment fat-free mass gain, it is possible that interactions can take place. Fourth, although all included studies were randomized and placebo-controlled trials; blinding, allocation concealment, quality of randomization, and details of withdrawals were not always reported. Fifth, only 10 studies were eligible for this meta-analysis, four of which were conducted in participants with specific medical conditions, such as sarcopenia, frailty, mobility-limited and obesity, which may limit the generalization of the findings. Despite these limitations, this meta-analysis provides a thorough overview of the previous published studies investigating the effects of milk proteins supplementation on body weight and composition during resistance exercise training in older adults.

Conclusions

This meta-analysis of RCTs provides evidence that supplementation of milk proteins, in conjunction with resistance training, is effective to elicit fat-free mass gain in older adults. However, it does not have significant effect on fat mass and body weight. Our findings suggest that milk proteins supplementation in combination with resistance training may play a role in preventing sarcopenia and age-related muscle loss. Hence, adopting a balanced diet with adequate protein along with exercise may contribute to sarcopenia prevention. In addition, more RCTs are needed to reveal the optimal dosage and type of protein, protein, the timing and frequency of protein supplementation, and type and frequency of resistance training.

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Table S1
Quality assessment of included RCTs in this meta-analysis

Study(year)	Selection bias		Performance bias	Detection bias
	Random sequence generation	Allocation concealment	Blinding of participants and study investigators	Blinding of outcome assessment
Campbell, 1995 (11)	Unclear	Unclear	Unclear	Unclear
Candow, 2006 (12)	Unclear	Unclear	Low risk	Low risk
Eliot, 2008 (22)	Unclear	Unclear	Low risk	Low risk
Verdijk, 2009 (23)	Unclear	Unclear	Unclear	Unclear
Kukuljan, 2009 (24)	Unclear	Unclear	Unclear	Unclear
Tieland, 2012 (13)	Low risk	Low risk	Low risk	Low risk
Chalé, 2013 (25)	Low risk	Low risk	Low risk	Low risk
Leenders, 2013 (26)	Unclear	Unclear	Low risk	Low risk
Verreijen, 2015 (27)	Low risk	Low risk	Low risk	Low risk
Rondanelli, 2016 (14)	Low risk	Low risk	Low risk	Low risk

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