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The impact of dietary protein or amino acid supplementation on muscle mass and strength in elderly people:

Individual participant data and meta-analysis of RCT's

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ABSTRACT

Objectives

Increasing protein or amino acid intake has been promoted as a promising strategy to increase muscle mass and strength in elderly people, however, long-term intervention studies show inconsistent findings. Therefore, we aim to determine the impact of protein or amino acid supplementation compared to placebo on muscle mass and strength in older adults by combining the results from published trials in a meta-analysis and pooled individual participant data analysis.

Design

We searched Medline and Cochrane databases and performed a meta-analysis on eight available trials on the effect of protein or amino acid supplementation on muscle mass and strength in older adults. Furthermore, we pooled individual data of six of these randomized double-blind placebo-controlled trials. The main outcomes were change in lean body mass and change in muscle strength for both the meta-analysis and the pooled analysis.

Results

The meta-analysis of eight studies (n=557) showed no significant positive effects of protein or amino acid supplementation on lean body mass (mean difference: 0.014 kg; 95% CI -0.152; 0.18), leg press strength (mean difference: 2.26 kg; 95% CI -0.56; 5.08), leg extension strength (mean difference: 0.75 kg; 95% CI: -1.96, 3.47) or handgrip strength (mean difference: -0.002 kg; 95% CI -0.182; 0.179). Likewise, the pooled analysis showed no significant difference between protein and placebo treatment on lean body mass (n=412: p=0.78), leg press strength (n=121: p=0.50), leg extension strength (n=121: p=0.16) and handgrip strength (n=318: p=0.37).

Conclusions

There is currently no evidence to suggest that protein or amino acid supplementation without concomitant nutritional or exercise interventions increases muscle mass or strength in predominantly healthy elderly people.

INTRODUCTION

Aging is associated with the loss of muscle mass and muscle strength, also referred to as sarcopenia [1]. This condition is associated with a decline in physical functioning leading to a higher risk of falls, fractures, and physical disability [2-4]. Muscle mass decline can be as high as 0.5% per year and strength is lost even more rapidly at a rate of 3% per year in elderly people [5]. Development and progression of sarcopenia are triggered by multiple factors, such as a sedentary lifestyle and inadequate dietary protein intake [6, 7]. Observational studies show that a high dietary protein intake is associated with a lower loss of lean body mass and less frailty compared to a low protein intake [7, 8]. Data from stable isotope studies show that the intake of dietary protein and/or amino acids stimulate muscle protein synthesis and decreases muscle protein breakdown, resulting in net muscle protein balance and muscle mass accretion [9, 10]. Although increasing dietary protein and amino acids intake seems to be a promising strategy to augment muscle mass, evidence from long-term, i.e. 3 to 12 months, intervention studies show inconsistent results [11-17]. While some studies show no effect [11, 12, 15], others present an increase in muscle mass after at least 3 months of protein supplementation [13, 14, 16, 17]. Similar discrepancy exists with muscle strength, where beneficial effects [15] or no differences between protein and placebo groups are observed [12, 16]. Therefore, we combined the results of randomized controlled trials to assess the effect of protein and amino acid supplementation without any concomitant nutritional or exercise intervention on muscle mass and strength in the elderly. In addition, we conducted a pooled analysis of those randomized controlled trials from which we were able to obtain individual participant data.

METHODS

This individual participant data analysis and meta-analysis were conducted in accordance with the recommendations and criteria as outlined in the Cochrane Handbook for Systematic Reviews of Interventions [18]. The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement was followed in the reporting of this systematic review [19].

Literature search and selection

We performed a literature search in Medline and the Cochrane Central Register of Controlled Trials (CENTRAL) in July 2016. For the databases, specific search terms were formulated, deducted from the eligibility criteria and linked to dietary protein supplementation, muscle mass and muscle strength. A complete overview of the search can be found in **Appendix 1**.

We included only randomized, double-blind, controlled human intervention trials that assessed the effects of protein and/or amino acid supplementation in elderly people (mean age ≥ 65 years, minimum age 50 years) on outcome variables related to muscle mass, one-repetition maximum (1-RM) leg strength or handgrip strength. Study inclusion for the outcome related to muscle mass were hydro densitometry (underwater weighing), bio impedance analysis (BIA), whole-body air plethysmography (BodPod), computed tomography (CT), magnetic resonance imaging (MRI) and dual energy X-ray absorptiometry (DXA). Study inclusion for the outcome related to strength were limited to three discrete measurements of maximal strength capacity, including handgrip strength and (double) leg 1-RM strength tests for leg press and/or leg extension. We only included studies with a minimum protein supplementation duration of seven days that were available in English full text. Cross-sectional studies, retrospective studies or studies published as letters, commentaries, editorials, case reports, reviews or duplicate publications from the same studies were excluded. In addition, studies that included concomitant intervention were excluded.

Use of data

Principal investigators of all eligible articles were invited to collaborate and to share individual participant data. Collaborators provided individual participant data on gender, age, weight, height, living status (free-living, prefrail, frail or institutionalized), underlying diseases, as well as baseline and follow-up measurements of muscle mass and/or 1-RM leg press and/or extension strength and/or handgrip strength. In addition, details on the protein amount, protein source, placebo and intervention duration were collected. We requested individual participant data for all participants that were randomized in the original study allowing us to perform an intention-to-treat analysis. The different data sets from all publications were synchronized and combined into one dataset. Data from eligible studies that were not available for the pooled analysis were extracted from the original publications. This extraction was performed in duplicate by two investigators (RF and MT). Differences in extracted data were resolved by group consultation (RF, MT and CD) until unanimous consensus was reached.

Risk of bias assessment

All studies were evaluated for risk of bias according to the recommendations of the Cochrane Collaboration [19] by two investigators (RF and MT) independently. One of the investigators (MT) authored one of the included studies [15], therefore two other investigators (RF and CD) evaluated this study. The items of this tool comprise a judgment and a support to assess whether the studies had a 'low risk', 'high risk' or 'unclear risk' of bias. Differences in opinion were resolved by group consultation (RF, MT and CD) until unanimous consensus was reached. To assess the risk of publication bias due to underrepresentation of studies with small sample sizes, Begg's funnel plots were visually inspected for each outcome variable (muscle mass, leg strength and handgrip strength). Additionally, Begg's and Egger's tests were assessed.

Statistical analysis

Meta-analysis

Combined estimates of dietary protein treatment effects were calculated by applying a random-effects meta-analysis model. Treatment effects were calculated using the mean changes (post-intervention subtracted from pre-intervention) and SD-change for each group. If SD-change was not available from the original published paper, we calculated the SD-change using the following equation: $SD\ change = \sqrt{[(SD_{pre})^2 + (SD_{post})^2 - 2 * corr(pre, post) * SD_{pre} * SD_{post}]}$ [20], where the correlation factor (corr) represents the mean of the available correlations from the pooled analysis. This resulted in correlation coefficients of 0.69 for the protein group and 0.67 for the placebo group for lean body mass, 0.97 for the protein group and 0.99 for the placebo group for leg extension strength, 0.99 for the protein group and 0.99 for the placebo group for leg press strength and 0.75 for the protein group and 0.70 for the placebo group for leg extension strength. Heterogeneity between studies was evaluated using the I^2 -statistic. The meta-analyses were performed using STATA Statistical Software (Release 14. StataCorp. 2016. College Station, TX: StataCorp LP) with statistical significance defined as $p < 0.05$.

Pooled analysis

For the individual participant data (IPD) analyses, baseline characteristics were analysed using an independent sample T-test. Differences between protein and placebo supplementation overtime were analysed using ANCOVA with 'treatment' as independent variable, 'end parameters' as dependent variable and 'baseline parameters' and 'study' as covariates. We included a variable named 'study' to take into account differences between trials. One trial was coded 1a and 1b representing 20% leucine group vs. placebo group and 40% leucine group vs placebo group, respectively [21]. The pooled analyses were performed using SPSS Statistics (version 22) with statistical significance defined as $p < 0.05$.

RESULTS

After removing duplicates, 1137 identified articles remained of which 1068 were excluded during review of title and abstract (**FIGURE 1**). Full texts of the remaining 69 articles were obtained and assessed, yielding eight applicable articles for the meta-analysis [11, 12, 15, 16, 21-24]. In total, we received individual participant data from six articles, which was used for the individual participant data analysis [11, 12, 15, 21-23]. Details of the eight trials are provided in **TABLE 1**.

Participants

The average age in the included studies ranged from 67 ± 1 to 88 ± 6 . In the pooled dataset ($n=486$), the mean age at baseline was 74.9 ± 5.1 years and 72.4% ($n=352$) of the participants was female. Participants in the pooled analysis were healthy ($n=282$) [11, 21, 23], sarcopenic ($n=78$) [22], frail ($n=65$) [15], diabetic ($n=67$) [12, 22] or had cancer ($n=1$) [23]. The two studies that were not integrated in the pooled analysis, since we were unable to receive the data, were conducted in healthy females [16] and residential care habitants [24].

Intervention

Participants randomly received protein, amino acids or placebo in all eight trials. The dose of protein or amino acid supplementation in the eight trials ranged from 6 to 30 grams per day (weighted mean 23.9 g/day) provided as a single amino acid (leucine), a mixture of essential amino acids (EAA), or milk-based protein. One trial used two types of mixtures of EAA, containing 20% and 40% leucine respectively [21]. The control interventions were health education, isocaloric and non-isocaloric placebo capsules or non-isocaloric carbohydrate containing drinks. The duration of the interventions ranged from 84 to 730 days.

Main outcome measures

In six of the articles [11, 12, 15, 16, 21, 23] DXA was used to measure muscle mass and in the other two articles BIA was used [22, 24]. Three studies measured 1-RM leg press [11, 12, 15], four studies measured 1-RM leg extension [11, 12, 15, 16] and six studies measured handgrip strength [15, 16, 21-24].

Publication bias

The risk of bias assessed by the Cochrane Collaboration recommendations was found to be low in the different trials.

Meta-analysis

The meta-analysis of 557 participants in the eight trials showed a combined weighted mean difference of 0.014 kg (95% CI -0.152; 0.18) for lean body mass (**FIGURE 2**). Furthermore, a meta-analysis of 165 participants in four trials that measured muscle strength also revealed no statistically significant differences in change in leg extension strength between protein vs. placebo (weighted mean difference: 0.75 kg (95% CI -1.96; 3.47) (**FIGURE 3**). A meta-analysis of 151 participants showed a combined weighted mean difference of 2.26 kg (95% CI -0.56; 5.08) for leg press strength (**FIGURE 4**). A meta-analysis of 471 participants showed a combined weighted mean difference of -0.002 kg (95% CI -0.182; 0.179) for handgrip strength (**FIGURE 5**). The heterogeneity in the meta-analysis of leg extension strength and leg press strength were rather high ($I^2=97.7\%$ and $I^2=97.4\%$ respectively, $p<0.00$).

Pooled analysis

The pooled analysis on 412 participants from six trials showed that the change in lean body mass was not significantly different between protein and placebo group ($p=0.78$) (**FIGURE 6**). In addition, the pooled analysis on 121 participants from three trials also did not show profound effects of protein supplementation on leg

press strength ($p=0.50$) or leg extension strength ($p=0.16$). Also, the pooled analysis on 318 participants from four trials did not show positive effects of protein or amino acid supplementation on handgrip strength ($p=0.37$). No sensitivity analyses or subgroup analyses were performed due to the limited number of available trials.

DISCUSSION

This combined analysis of eight randomized controlled trials and the IPD analysis of six trials showed no difference between protein or amino acid supplementation as opposed to placebo supplementation on lean body mass, leg strength or handgrip strength in elderly people.

Our results are in line with the meta-analysis of Xu et al. [25] on lean body mass and strength, but in contrast with the meta-analysis of Komar et al. [26] on lean body mass. The apparent discrepancy is caused by differences in inclusion and exclusion criteria. Unlike the studies of Xu et al. and Komar et al., we excluded studies that provided a concomitant exercise/physical activity or nutrition intervention. This enabled us to exclusively observe the effects of protein or amino acid intake on muscle mass and strength in elderly people without the potential concurrent effects of exercise [9] and other macronutrients [27, 28] or micronutrients [29, 30] present in the intervention or placebo supplements. An additional strength of our article is that we had access to the original data of six studies which enabled us to pool the data and standardize the outcome variables across the studies.

The present study does not show beneficial effects of protein or amino acid supplementation on lean body mass or strength in elderly people. These findings may be explained by the amount and source of dietary protein supplementation which varied among the included studies. The amount of protein ranged from 6 [22] to 30 [15, 23] g of protein per day and half of the studies (n=4) provided ≤ 7.5 g protein and or amino acids per day [11, 12, 22]. It is equivocal if this amount is sufficient to augment muscle mass gain in elderly. Data suggests that the post-prandial muscle protein synthetic response to smaller, meal-like amounts of amino acids is attenuated in older subjects [31, 32], and that 25-30 g of protein per main meal is needed to maximize muscle protein synthesis in the elderly [33, 34]. This suggests that adding 7.5 g per day protein on top of the normal diet may be insufficient to improve muscle mass gain and strength in the elderly [35] and more protein may be

needed. One study, however, increased protein intake up to 25-30 g per main meal and still demonstrated no benefits on muscle mass [15], but more randomized intervention trials are needed to confirm this.

In addition to the amount of protein also the source of protein might be important to improve muscle mass and strength in the elderly. Studies included in our analysis provided either a single amino acid (leucine) [11, 12], a mixture of EAA's [16, 21, 22], or milk-based protein [15, 23]. Even though all protein sources have the capacity to stimulate muscle protein synthesis, the postprandial muscle protein fractional synthetic response can vary substantially between different protein sources [35]. The differences in anabolic response between protein sources may be explained by the digestion and absorption kinetics as well as the composition of amino acids. Single amino acids or EAA's may be rapidly digested which strongly increase the postprandial plasma amino acid concentrations and, as such, stimulate muscle protein synthesis in the elderly. Intact protein may delay protein digestion and absorption. However, a recent study showed that this delay of milk based protein digestion and absorption does not negatively modulate postprandial muscle protein synthesis rates in older men [36]. Also the composition of EAA's may be different between sources. In the studies included, the protein supplements consist of high quality protein sources rich in EAA's and it is unlikely that the sources used in various trials differ in the ability to stimulate muscle protein synthesis in the elderly.

An important factor that might explain our results might be the habitual dietary protein or amino acid intake of elderly people. Unfortunately not all our included trials did collect dietary intake data, however, based on five studies, the average habitual protein intake was 1.0 g protein per kg-bw/d [11, 12, 15, 21, 23]. The latter protein intake might be too high to observe any additional effects of dietary protein or amino acid supplementation on muscle mass or strength in older adults. Dietary protein supplementation might be more effective in elderly consuming less than the recommended daily allowance of 0.83 g/kg-bw/d [37]. Protein supplementation research with a focus on malnourished elderly might clarify the matter.

Another difference between the included studies in our analyses is the duration of the intervention. We included eight studies ranging in duration between 12 to 104 weeks. The majority of studies (i.e. seven out of eight) lasted ≤ 24 weeks which may be too short to observe any measurable changes in muscle mass and strength in elderly. In people aged 65 years and older, the yearly loss of muscle mass is estimated to be ~ 0.2 kg [38, 39]. Translating this to an intervention study with a duration of ≤ 24 weeks, a loss of 0.05 to 0.1 kg muscle mass is to be expected in the placebo group. Considering the measurement error of the DXA and BIA (CV of lean tissue $< 0.5\%$), such small differences in changes between protein vs. placebo may not be detected. A more prolonged intervention, e.g. 3 years in which muscle loss in the placebo group would be estimated at ~ 0.6 kg would result in a larger difference in changes between protein vs. placebo which is more likely to be measurable with DXA and BIA. In line with this, observational evidence suggests that higher intake of dietary protein prevents the age-related loss of muscle mass after 3 years [7]. Therefore, if practically feasible, it may be interesting to investigate the effect of protein supplementation in elderly people over a longer time period.

One could speculate that a potential benefit of dietary protein supplementation is more evident in very frail or hospitalized elderly as compared with apparently healthy people. The difference in efficacy of protein supplementation to augment muscle mass might be attributed to differences in amount of muscle mass, inflammatory status, hormone levels, insulin resistance, the level of habitual physical activity, as well as habitual dietary protein intake [4, 40]. In the present analysis, the majority of studies included elderly that were apparently healthy and those studies that included frail elderly were too limited to permit a subgroup analysis. Clearly more research is warranted to investigate the impact of protein or amino acid supplementation on muscle mass or strength outcomes in very frail and hospitalized elderly people.

Our aim was to provide evidence of protein supplementation on muscle mass and muscle strength without a concomitant intervention such as physical activity. Physical activity is the most potent stimuli to increase muscle protein synthesis rates. Physical activity sensitizes skeletal muscle tissue allowing better uptake of

dietary protein in the muscle to increase muscle protein synthesis rates and muscle mass gain [9]. Earlier studies showed that protein supplementation augmented the adaptive response of skeletal muscle to resistance-type exercise training in both young, healthy and frail elderly [20, 41, 42]. These data clearly show that the combination of physical activity and adequate protein ingestion is a promising strategy to augment muscle hypertrophy and treat sarcopenia in elderly people.

CONCLUSION

There is currently no evidence to conclude that protein or amino acid supplementation without concomitant exercise or nutritional interventions increases muscle mass or muscle strength in the elderly.

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1 **TABLE 1:** Study details and participant characteristics of the eight included trials

		Health status	Age (years)	Gender (m/f)	Treatment	Protein amount (g/day)	Iso caloric	Duration of trial (days)	Included in pooled analysis
Carlsson et al. (2011)	Protein (n=47)	ADL-dependent	88 ± 6	13/34	Milk based	7.4	No	84	no
	Placebo (n=47)		85 ± 7	11/36	CHO	0			
Dillon et al. (2009)	Protein (n=7)	Healthy	67 ± 1	0/7	EAA	15	Unknown	90	no
	Placebo (n=7)		69 ± 3	0/7	PLA	0			
Kim et al. (2012)	Protein (n=39)	Sarcopenic	79 ± 3	0/39	EAA	6	No	90	yes
	Placebo (n=39)		79 ± 3	0/39	HE	0			
Leenders et al (2012)	Protein (n=30)	Healthy	71 ± 5	30/0	Leucine	7.5	Unknown	168	yes
	Placebo (n=30)		70 ± 4	30/0	Wheat flower	0			
Tieland et al. (2012)	Protein (n=34)	Frail	78 ± 8	14/20	Milk based	30	no	168	yes
	Placebo (n=31)		81 ± 7	16/15	PLA	0			
Verhoeven et al. (2009)	Protein (n=15)	Diabetic	71 ± 4	15/0	Leucine	7.5	Unknown	84	yes
	Placebo (n=15)		71 ± 5	15/0	Wheat flower	0			
Ispoglou et al. (2016) 1a	Protein (n=8)	Healthy	71 ± 3	3/5	EAA (20% leucine)	15	Yes	90	yes
	Placebo (n=9)		72 ± 3	4/5	PLA (lactose)	0			
Ispoglou et al. (2016) 1b	Protein (n=8)	Healthy	72 ± 3	4/4	EAA (40% leucine)	15	Yes	90	yes
	Placebo (n=9)		72 ± 3	4/5	PLA (lactose)	0			

Zhu et al. (2015)	Protein (n=10)	Healthy	74 ± 3	0/101	skim milk + whey protein isolate	30	Yes	730	yes
	Placebo (n=95)		74 ± 3	0/95	skim milk-based + CHO	2.1			

2 Abbreviations: ADL= activities of daily living, CHO = Carbohydrate, EAA = essential amino acids, PLA= Placebo, HE=health education