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EFFECTS OF LEUCINE-RICH PROTEIN SUPPLEMENTS ON ANTHROPOMETRIC PARAMETER AND MUSCLE STRENGTH IN THE ELDERLY: A SYSTEMATIC REVIEW AND META-ANALYSIS

B. KOMAR, L. SCHWINGSHACKL, G. HOFFMANN

Department of Nutritional Sciences, Faculty of Life Sciences, University of Vienna, Althanstreet 14 (UZAII), A-1090 Vienna, Austria. Corresponding author: Georg Hoffmann, PhD, Department of Nutritional Sciences, Faculty of Life Sciences, University of Vienna, Althanstreet 14 (UZAII), A-1090 Vienna, Austria, M: georg.hoffmann@univie.ac.at

Abstract: *Objective:* The primary objective of the present systematic review and meta-analysis was to synthesize the available literature relating to leucine supplementation in the elderly with respect to its effects on anthropometrical parameters and muscle strength. The secondary aim was to perform a selective subgroup analysis when possible differentiating between healthy and sarcopenic subjects. *Methods:* Literature search was performed using the electronic databases MEDLINE, EMBASE, SportDiscus, and the Cochrane Central Register of trials with restrictions to randomized controlled trials or studies following a cross-over design. Parameters taken into account were body weight, body mass index, lean body mass, fat mass, percentage of body fat, hand grip strength, and knee extension strength. Moreover, biomarkers of glucose metabolism (fasting glucose, fasting insulin, albumin, and HOMA index) were extracted when possible. For each outcome measure of interest, a meta-analysis was performed in order to determine the pooled effect of the intervention in terms of weighted mean differences between the post-intervention (or differences in means) values of the leucine and the respective control groups. Data analysis was performed using the Review Manager 5.2.4. software. *Results:* A total of 16 studies enrolling 999 subjects met the inclusion criteria. Compared with control groups, leucine supplementation significantly increased gain in body weight [mean differences 1.02 kg, 95%-CI (0.19, 1.85), p=0.02], lean body mass [mean differences 0.99 kg, 95%-CI (0.43, 1.55), p=0.0005], and body mass index [mean differences 0.33 kg/m2, 95%-CI (0.13, 0.53), p=0.001], when compared to the respective control groups. With respect to body weight and lean body mass, leucine supplementation turned out to be more effective in the subgroup of study participants with manifested sarcopenia. All other parameters under investigation were not affected by leucine supplementation in a fashion significantly different from controls. *Conclusions:* It is concluded that leucine supplementation was found to exert beneficial effects on body weight, body mass index, and lean body mass in older persons in those subjects already prone to sarcopenia, but not muscle strength. However, due to the heterogeneity between the trials included in this systematic review, further studies adopting a homogenous design with respect to participant characteristics duration as well as the kind and amount of daily supplement in use are required.

Key words: Leucine, sarcopenia, lean body mass, aging, meta-analysis.

Introduction

There is a worldwide increase in the proportion of the elderly or retired population. According to the World Health Organization, the number of people aged 60 years or older will rise between the year 2000 and 2050 from actually 600 million to more than two billion, equal to a duplication of their share of the total population from 11% to 22%.

Ageing is associated with a number of physical changes, one of them being sarcopenia. This process is characterized by a progressive loss of muscle mass and muscle strength (1). While lean body mass (LBM) contributes to approximately 50% of total body weight in younger adults, the respective value decreases to some 25% by the age of 75 years (2). Depletion of muscle mass is accompanied by higher risks of bone fractures and the development of diseases such as type 2 diabetes (1). To date, there is still no precise concept for the primary and secondary prevention of sarcopenia. Apart from regular physical activity, diet is generally accepted to have a considerable impact on muscle protein synthesis. Among the macronutrients, protein is considered to exert the strongest stimulus on muscle growth. Special emphasis is placed on branched-chain amino acids (BCAA) such as leucine (3-5). Speed and extent of digestion and absorption of dietary protein do not seem to differ between young and older adults (6, 7). However, the magnitude of muscle protein synthesis induced by dietary amino acids is restricted in the elderly (8, 9). This condition has been termed anabolic resistance. Leucine is known to stimulate muscle protein synthesis in both an insulin-dependent and an insulin-independent fashion. Thus, it seemed reasonable to speculate that anabolic resistance might be compensated via an increased uptake of dietary leucine (10-12).

In a number of in vitro studies, BCAA as well as leucine were shown to have anabolic effects on skeletal muscle tissue (13, 14), and ensuing animal experiments could confirm these findings (15, 16). In addition, short-term interventions in humans further indicate an advantageous effect of leucine supplementation. Following an intravenous application of the amino acid, both an increase in muscle protein synthesis (17, 18) and an attenuation of muscle protein breakdown were reported (19, 20). Comparable benefits could be observed in

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experiments applying amino acid formulas per os (21-23). However, studies investigating long-term effects of leucine supplementation on muscle mass and muscle strength in senior volunteers provided heterogeneous results. Thus, Borsheim et al. (24) observed an increase in muscle strength in seniors due to a 16-week supplementation with essential amino acids (corresponding to an additional uptake 5.6 g/d leucine). In contrast, Verhoeven et al. (25) did not find any effects of leucine supplements (8-15 g/d) in senior volunteers. There is no clear explanation for the discrepancy between these findings and it remains difficult to evaluate whether leucine supplementation has any benefit on the gain in muscle mass and/or strength in the elderly. The primary objective of the present study was to analyze the available literature relating to this topic in a quantitative way via meta-analyses. The secondary aim was to perform a selective subgroup analysis when possible differentiating between healthy and sarcopenic subjects.

Methods

Search strategy

Queries of literature were performed using the electronic databases MEDLINE (between 1966 and February 2014), EMBASE (between 1980 and February 2014), SportDiscus (until February 2014), and the Cochrane Central Register of trials (until February 2014) with restrictions to randomized controlled trials or studies following a cross-over design, but no restrictions to language and calendar date using the following search term: „leucin* OR isoleucin* OR valin* OR bcaa* OR branched chain amino acid* OR branched-chain amino acid* OR essential amino acid* OR eaa*“ in combination with “protein OR whey* OR milk*”. Moreover, the reference lists from retrieved articles were checked to search for further relevant studies, and systematic reviews and meta-analysis were searched. This systematic review was planned, conducted, and reported in adherence to standards of quality for reporting meta-analyses (26).

Study selection

Studies were included in the meta-analysis if they met all of the following criteria: (1) RCTs or studies with a cross-over design; (2) minimum intervention period of 10 days; (3) enrolling volunteers aged 65 years or older; (4) supplementation with leucine of at least 2 g/day in accordance with data proposed by the pertinent literature (9, 12, 27); (5) assessment of anthropometric parameters: body weight (BW) or body mass index (BMI) or lean body mass (LBM) or fat mass (FM) or percentage of body fat; (6) assessment of parameters of muscle strength: hand grip strength or knee extension strength; (7) assessment of parameters of glucose metabolism: fasting glucose (FG) or fasting insulin (FI) or plasma albumin or HOMA index; (8) studies enrolling patients with chronic diseases (e.g. chronic obstructive pulmonary disorders, cancer, renal insufficiency with hemodialysis) were excluded. In addition, trials using training or exercise groups as controls in comparison to leucine supplementation were excluded. However, when physical activity was part of the design in both leucine and control groups, studies were included if the other criteria were fulfilled. If data of ongoing studies were published as updates, results of only the longest duration periods were included.

Risk of Bias Assessment

Full copies of studies were independently assessed for methodological quality by all authors using the Risk of bias assessment tool by the Cochrane Collaboration. The following sources of bias were detected: selection bias, performance/ detection bias attrition bias, reporting bias and other bias (28, 29) (Figure 1).

Data Extraction and statistical analysis

The following data were extracted from each study: the first author’s last name, publication year, study duration, number of volunteers, participant´s sex and age, BMI, amount of leucine used for supplementation, outcomes and post mean values (if not available change from baseline values were used) with corresponding standard deviation. For each outcome measure of interest, a meta-analysis was performed in order to determine the pooled effect of the intervention in terms of weighted mean differences (MDs) between the post-intervention (or differences in means) values of the leucine and the respective control groups. Combining both the post-intervention values and difference in means in one meta-analysis is a legitimate method described by the Cochrane Collaboration (30). All data were analyzed using the software REVIEW MANAGER 5.2.4. as provided by the Cochrane Collaboration (http://ims.cochrane.org/revman). Forest plots were generated to illustrate the study-specific effect sizes along with a 95%-CI. Heterogeneity between trial results was tested with a standard $\chi 2$ test. The I2 parameter was used to quantify any inconsistency: $I2 = [(Q - d.f.)]/Q \times 100\%$, where Q is the $\chi 2$ statistic and d.f. is its degrees of freedom. A value for $I2 > 50\%$ was considered to represent substantial heterogeneity (31).

Funnel plots were used to assess potential publication bias (e.g. the tendency for studies that yield statistically significant results to be more likely to be submitted and accepted for publication). To determine the presence of publication bias, we assessed the symmetry of the funnel plots in which mean differences were plotted against their corresponding standard errors. Data extraction was conducted independently by all authors, with disagreements resolved by consensus.

Missing data

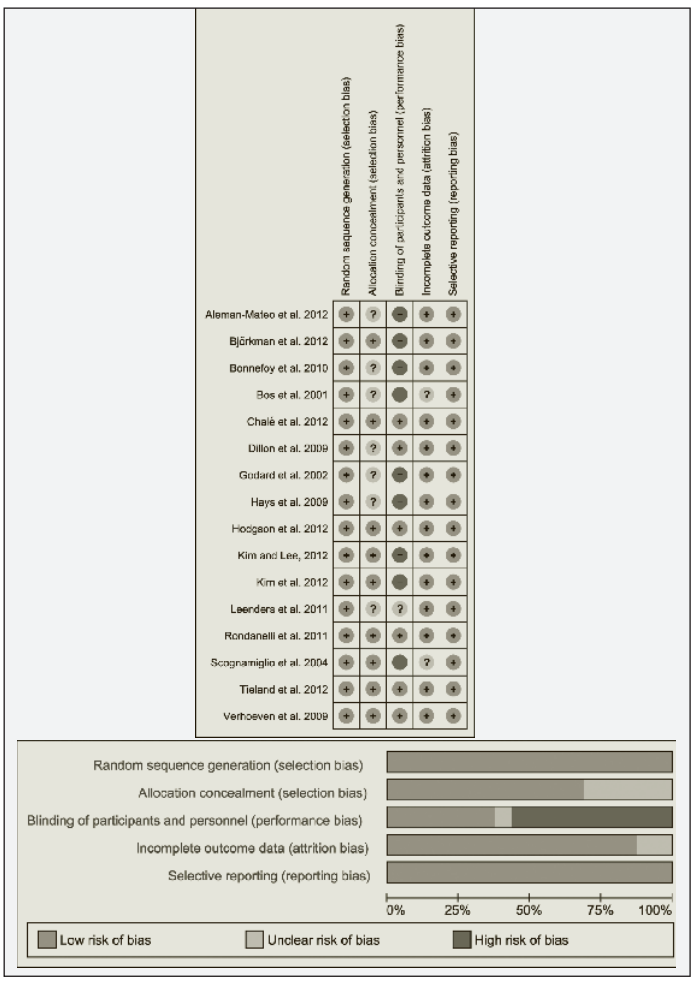
Data processing for this review required the input of the mean and standard deviation (SD) of post-intervention values or differences in means. When SD was not available, standard errors and confidence intervals for means were used

to calculate standard deviations, according the guidelines by the Cochrane Handbook (28). In one case (32), no data were provided and the median was assumed to be identical to the mean value while SD was calculated using the formula range/6 described by Hozo et al. (33) as a suitable method for studies with a number of cases higher than 70.

When the exact amount was not given, leucine content of the formula was calculated using either the German Nutrient Database provided by the software package nut.s (dato Denkwerkzeuge, Vienna, Austria) or by contacting the corresponding authors of the respective studies.

Figure 1

Risk of bias assessment tool. Across trials, information is either from trials at a low risk of bias (green), or from trials at unclear risk of bias (yellow), or from trials at high risk of bias (red). For each study, every bias domain will be checked, the given summary represents an assessment of bias risk across studies. For each bias domain, low risk of bias means that information is from studies at low risk of bias, high risk of bias indicates the proportion of information from studies at high risk of bias which might be sufficient to affect the interpretation of the results, and unclear risk of bias refers to information from studies at low or unclear risk of bias



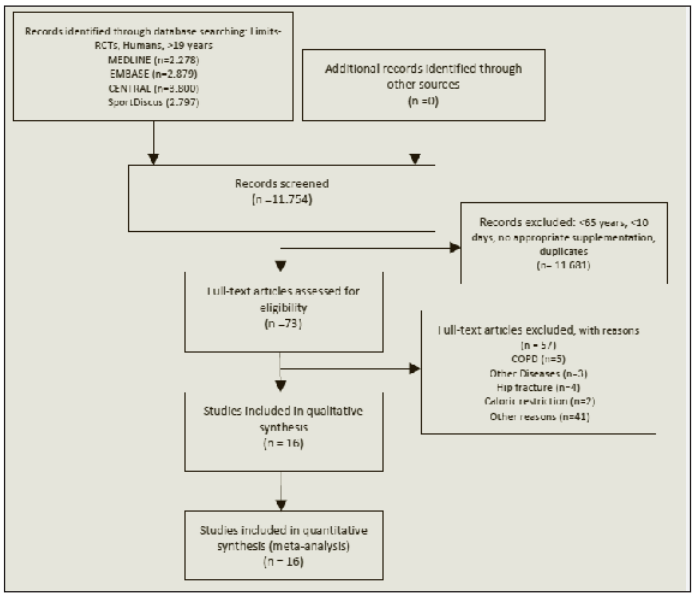
Results

Literature search and study characteristics

A total of 16 studies enrolling 999 subjects extracted from 11.754 articles met the inclusion criteria and were enclosed for meta-analyses (25, 32, 34-47). The detailed steps of the systematic article search and selection process are given as a flow chart in Figure 2. Ten studies with a total of 602 participants were enrolling healthy volunteers, while the other 6 studies (n=397 subjects) were enrolling senior volunteers with sarcopenia. Sample size varied between 11 and 181 participants with a study duration ranging from 10 days to two years. Additional amount of leucine varied between 2 g/d and 7.8 g/d and was supplied either via amino acid formulas (BCAA, essential amino acids, n=6 studies), whey protein (n=4 studies), casein protein (n=2 studies), ricotta cheese (n= 1 study) or commercially available protein-enriched energy drinks (n=3 studies). General study characteristics are summarized in Table 1.

Figure 2

Flow chart for meta-analysis article selection process. COPD = chronic obstructive pulmonary disease



Anthropometric parameters

Compared with control groups, leucine supplementation significantly increased gain in BW [mean differences (MD) 1.02 kg, 95%-CI (0.19, 1.85), p=0.02]. Subgroup analyses showed that leucine supplementation had no effects in healthy seniors [MD 0.35 kg, 95%-CI (-1.90, 2.61), p=0.76), whereas increase in BW turned to be significantly more pronounced following leucine supplementation in the subgroup of study participants with sarcopenia [MD 0.75 kg, 95%-CI (0.22, 1.28), p=0.005) (Figure 3).

MD in change of LBM [0.99 kg, 95%-CI (0.43, 1.55),

Table 1A
General study characteristics

Reference	Number and characteristics of volunteers	Age % female % male	Study length	Reports on energy intake (kcal/d; mean ± SD*)	Protein supplementation characteristics	Leucine content in supplement
Aleman-Mateo et al. 2012	40 Sarcopenia	76 57.5% female 42.5% male	3 months	n.d.	Ricotta cheese vs. control	210 g/d ricotta cheese 2 g/d leucine
Björkman et al. 2012	106 Nursing home residents	83.55 75.5 24.5	6 months	n.d.	Fruit juice vs. fruit juice + Whey (75% ß-lactoglobulin, 25% α-lactalbumin): 20g/d	2 g/d leucine
Bonnefoy et al. 2010	30 Undernutrition, catabolic status (MNA score <24)	80.95 57.1 female in control 61.5 female in supplement	2 weeks	Supplement: initial: 1219 ± 271 protein end: 1247 ± 348 Control: initial: 971 ± 202 end: 1118 ± 314	Protein-enriched vs. usual, balanced diet, PE: Hyperprotidine	<50 kg: 3,615 g leucine, 11.025 g protein 50-59 kg: 4.082 g leucine, 14.7 g protein >60 kg: 6.025 g leucine, 18.375 g protein
Bos et al. 2001	23 Geriatric, hospitalized, malnourished	78 56% female 43% male	10 days	Increased energy intake in supplement group: + 32%	Protein-enriched vs. control PE: Nutrigil hp danone	30 g/d milk protein 2.3 g/d leucine
Chalé et al. 2012	80 Mobility-limited	77.65 59% female 41% male	6 months	Supplement: initial: 1639 ± 376 end: 1464 ± 309 Control: initial: 1756 ± 540 end: 1580 ± 488	WPC vs. carbohydrate control	40 g/d protein: 4.8 g/d leucine
Dillon et al. 2009	14 healthy	68 100% female	3 months	n.d.	EAA vs. placebo	15 g/d EAA: 2.78 g/d leucine
Godard et al. 2002	17 Older men	71.5 100% male	12 weeks	Supplement: initial: 2609 ± 86 (SEM) end: 2350 ± 29 (SEM) Control: initial: 2229 ±32 (SEM) end: 2304 ± 72 (SEM)	AA + carbohydrate vs. control	12 g/d EAA: 2.24 g/d leucine
Hays et al. 2009	11 healthy	71.3 100% female	15 days	Isocaloric diet	Whey protein vs. collagen protein	38 g/d whey protein: 4.066 g/d leucine 38 g/d collagen protein: 1.178 g/d leucine
Hodgson et al. 2012	181	74.3 100% female	2 years	Isocaloric supplement	Whey protein vs carbohydrate placebo	30 g/d whey protein: 3.39 g/d leucine
Kim and Lee, 2012	84 Low income, frail	78.65 79.35% female 20.65% male	12 weeks	Supplement: initial: 965 ± 309 end: 1124 ± 315 Control: initial: 951 ± 331 end: 896 ± 277	Protein-enriched vs. control	5.64 g/d leucine + 2.192 g/d leucine (PE) = 7.832 g/d leucine (total)
Kim et al. 2012	155 Sarcopenia	79.1 100% female	3 months	n.d.	Exercise + AA vs. Exercise vs. AA vs. health education (control)	6 g/d AA: 2.52 g/d leucine
Leenders et al. 2011	57 Diabetes mellitus type 2	71 100% male	24 weeks 8 weeks	n.d. Supplement: initial: 1555 ± 215	Leucine vs. placebo EAA vs. placebo	7.5 g/d leucine 8 g/d EAA: 2.5 g/d leucine
Rondanelli et al. 2011	41 healthy	81.7 59% female 41% male		end: 1668 ± 339 Control: initial: 1920 ± 350 end: 1800 ± 273		
Scognamiglio et al. 2004	65 DM2	65.5 29% female 71% male	12 weeks treatment, 1 week washout, crossover Total study length: 25 weeks	n.d.	AA sup vs. placebo	12 g/d AA: 3.8 g/d leucine
Tieland et al. 2012	65 frail	65 55% female 45% male	24 weeks	Supplement: initial: 8.1 ± 0.4 (SEM) MJ/d end: 7.5 ± 0.4 (SEM) MJ/d Control: initial: 8.1 ± 0.4 (SEM) MJ/d end: 7.8 ± 0.4 (SEM) MJ/d	Casein protein vs. placebo	Casein:whey-ratio as in milk (30 g/d protein): 2,8 g/d leucine
Verhoeven et al. 2009	30 healthy	71 0% female 100% male	12 weeks	8.2 MJ/d, no difference between groups	Leucine vs. placebo	7.5g/d leucine

Table 1B

Baseline data of anthropometrical parameters, biomarkers of glucose metablosim, and muscle strength

		BW (kg)	LBM (kg)	BMI (kg/m2)	FM (kg)	%BF	FBG (mmol/L)	FI (mU/L)	HOMA	Albumin (g/l)	Hand Grip Strength (pounds/inch²)	Knee Extension Strength (Nm/kg)
Aleman-Mateo et al. 2012	Sup	68.7±8.6	37,1±6.3	26.5±4.0	28.8±3.6	41.9±9.4	5.3±0.64	9.9±3.8	2.4±1.2	-	-	-
	Con	67.8±10.9	36.9±6.4	26.1±3.7	28.0±4.5	41.3±10.1	5.4±0.62	9.5±6.2	2.3±1.8	-	-	-
Björkman et al. 2012	Sup	-	39.4±9.0	24.8±4.3	-	-	4.8±0.88	4.6±2.7	-	35.1±3.5	11.2±5.6	132±48
	Con	-	39.6±9.3	24.0±5.5	-	-	4.7±0.52	5.2±14.8	-	35.3±4.2	12.9±7.6	154±59
Bonnefoy et al. 2010	Sup	58.4±11.0	40.9±8.1	22.6±3.8	22.2±5.5	-	-	-	-	26.6±4.7	-	-
	Con	59.6±14.9	39.5±6.8	23.4±5.0	19.6±10.9	-	-	-	-	24.7±3.3	-	-
Bos et al. 2001	Sup	56.1±9.4	36.7±5.4	20.6±3.9	17.8±6.5	31.1±8.1	-	-	-	35.9±5.1	12.5±9.9	-
	Con	53.4±5.1	38.3±9.7	20.0±2.7	13.3±4.8	25.4±9.4	-	-	-	36.0±5.2	20.0±11.4	-
Chalé et al. 2012	Sup	73.0±10.8	46.7±8.6	27.0±3.2	25.9±6.9	-	-	-	-	-	-	-
	Con	73.8±11.2	46.4±8.4	26.9±3.1	25.6±7.2	-	-	-	-	-	-	-
Dillon et al. 2009	Sup	73±15.8	43.5±7.4	-	-	38±5.3	-	5.5±5.3	-	-	-	-
	Con	71±10.6	40.7±6.3	-	-	40±2.6	-	6.1±4.2	-	-	-	-
Godard et al. 2002	Sup	91.0±13.9	-	-	-	-	-	-	-	-	-	178±36.8
	Con	75.4±14.1	-	-	-	-	-	-	-	-	-	172±36
Hays et al. 2009	Sup	75.3±10. 5	41.9±3.3	29.2±3.9	-	43.7±6	-	-	-	-	-	-
	Con	75.0±10.2	42.1±3	29.2±3.9	-	43.2±6	-	-	-	-	-	-
Hodgson et al. 2012	Sup	67.4±11.1	-	26.3±3.8	-	-	5.3±0.49	7.18	2.1±0.9	-	-	-
	Con	69.7±11.5	-	27.2±3.9	-	-	5.3±0.5	8.05	1.9±0.9	-	-	-
Kim and Lee 2013	Sup	47.4±9.3	-	-	-	-	-	-	-	-	15.3±4.6	-
	Con	44.4±7.7	-	-	-	-	-	-	-	-	16.3±5.0	-
Kim et al. 2012	Sup	40.1±3.2	28.8±2.0	18.9±1.6	-	-	-	-	-	-	-	1.15±0.25
	Con	40.4±3.9	29.3±2.4	18.8±1.7	-	-	-	-	-	-	-	1.14±0.26
Leenders et al. 2011	Sup	83.6±9.7	61.9±5.9	27.4±3.2	19.0±4.3	22.5±3.2	8.0±1.0	13.16±4.4	4.7±1.6	-	-	205±37.0
	Con	84.6±10.6	62.2±6.9	27.2±3.2	19.6±5.3	22.9±4.2	7.5±1.0	14.27±5.1	4.8±2.1	-	-	202±37.7
Rondanelli et al. 2011	Sup	60.8±5.9	-	21.8±2.3	-	-	4.2±0.4	-	2.3±0.9	38.8±3	18.68±1.36	-
	Con	64.1±8.9	-	22.2±2.6	-	-	4.48±0.4	-	2.2±1.1	38.8±4	18.46±1.14	-
Scognamiglio et al. 2004	Sup	-	-	27.7±3.7	-	-	8.6±2.4	16±9	-	-	-	-
	Con	-	-	27.7±3.7	-	-	8.6±2.4	16±9	-	-	-	-
Tieland et al. 2012	Sup	73.9±13.9	45.8±9.9	-	25.3±8.7	-	5.2±0.6	18±7	-	-	26±11.6	57±29.2
	Con	73.8±12.2	46.7±9.5	-	23.9±8.3	-	5.3±0.5	18±6.7	-	-	26±11.1	57±27.8
Verhoeven et al. 2009	Sup	77.6±8.9	54.6±3.9	25.9±2.3	20.0±5.4	25.3±4.6	5.68±0.7	6.73±2.6	1.7±0.8	-	-	85±11.6
	Con	78.1±9	55.8±3.4	26.3±2.25	19.8±6.3	24.5±4.6	5.54±0.41	6.04±2.8	1.5±0.7	-	-	85±11.2

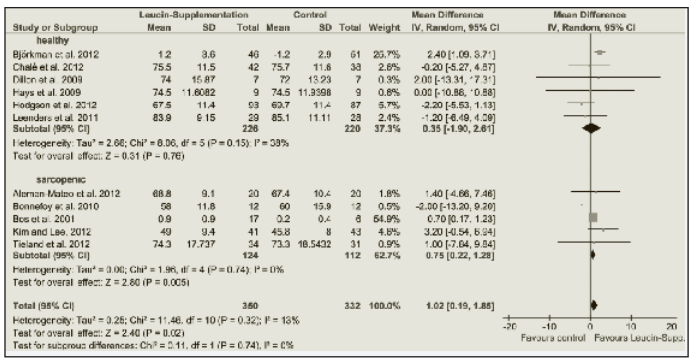
%BF = percent body fat; BW = body weight; BMI = body mass index; Con = control; FBG = fasting blood glucose; FM = fat mass; HOMA = homeostasis model assessment; LBM = lean body mass; Sup = supplement.

p=0.0005] were significantly more distinct in subjects supplemented with leucine as compared to control groups. Again, subgroup analysis revealed no significant changes between control and leucine groups in healthy subjects [MD -0.05 kg, 95%-CI (-1.55, 1.46), p=0.95], while in subjects with sarcopenia, increase in LBM was significantly more prominent following leucine supplementation as compared to controls [MD 1.14 kg, 95%-CI (0.55, 1.74), p=0.0002] (Figure 4).

BMI was significantly more enhanced in the leucine groups as compared to controls [MD 0.33 kg/m2, 95%-CI (0.13, 0.53), p=0.001], however, this was not reflected by significant changes in either healthy [MD -0.16 kg/m2, 95%-CI (-1.13, 0.82), p=0.75] or sarcopenic subgroups [MD 0.22 kg/m2, 95%-CI (-0.23, 0.67), p=0.33] (Figure 5).

Figure 3

Forest plot showing pooled MD with 95%-CI for body weight (kg) for 11 randomized controlled leucine supplementation studies. For each study, the shaded square represents the point estimate of the intervention effect. The horizontal line joins the lower and upper limits of the 95%-CI of these effects. The area of the shaded square reflects the relative weight of the study in the respective meta-analysis. The diamond at the bottom of the graph represents the pooled MD with the 95%-CI for the 11 study groups. Supp = supplementation



In contrast to these findings, leucine supplementation did not affect any other anthropometric measure (FM, percentual body fat) when compared to control groups (Table 2).

Parameters of glucose metabolism

Mean differences in change of FG, FI, HOMA index, and plasma albumin turned out to be not significantly different between leucine supplementation and control groups (Table 2).

Parameters of muscle strength

As with parameters of glucose metabolism, neither hand grip strength nor knee extension strength were affected by leucine supplementation in a fashion significantly different from control interventions (Table 2).

Figure 4

Forest plot showing pooled MD with 95%-CI for lean body mass (kg) for 10 randomized controlled leucine supplementation studies. For each study, the shaded square represents the point estimate of the intervention effect. The horizontal line joins the lower and upper limits of the 95%-CI of these effects. The area of the shaded square reflects the relative weight of the study in the respective meta-analysis. The diamond at the bottom of the graph represents the pooled MD with the 95%-CI for the 10 study groups. Supp = supplementation

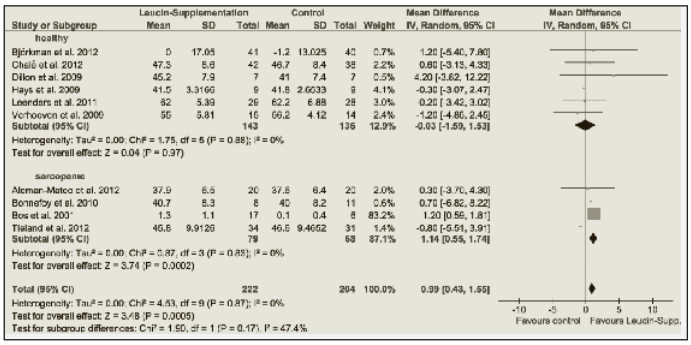
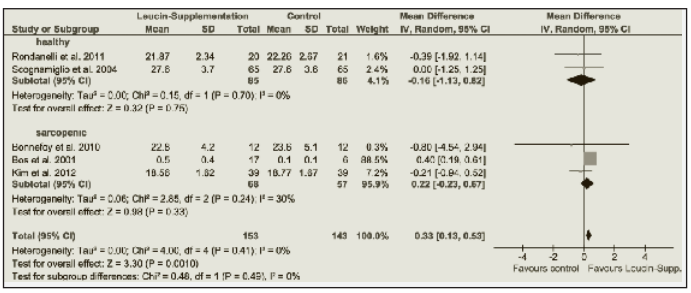


Figure 5

. Forest plot showing pooled MD with 95%-CI for body mass index (kg/m2) for 5 randomized controlled leucine supplementation studies. For each study, the shaded square represents the point estimate of the intervention effect. The horizontal line joins the lower and upper limits of the 95%-CI of these effects. The area of the shaded square reflects the relative weight of the study in the respective meta-analysis. The diamond at the bottom of the graph represents the pooled MD with the 95%-CI for the 5 study groups. Supp = supplementation



Discussion

In the present meta-analyses, leucine supplementation exerted a beneficial effect on body weight, body mass index, and lean body mass especially in senior volunteers with sarcopenia. With regard to the other parameters under investigation, leucine supplements were not superior to control interventions. This seems to be in contrast to other systematic reviews reporting an increase in hand grip strength (48) or in maximal leg press weight (49) following an additional

Table 2
Results of random effects meta-analyses of randomized controlled leucine supplementation studies

Outcome	WMD			95%-CI			P-Value			I^2 (%)		
Anthropometrical parameters	**T (n)**	**H (n)**	**S (n)**	**T**	**H**	**S**	**T**	**H**	**S**	**T**	**H**	**S**
Fat mass (kg)	-0.51 (7)	-0.53 (3)	-0.29 (4)	(-1.04, 0.02)	(-1.08, 0.02)	(-2.16, 1.59)	0.02	0.06	0.77	0	0	0
Percentual body fat (%)		-0.57 (4)	n.d.		(-2.27, 1.12)	n.d.		0.51	n.d.		0	
Glucose metabolism parameters												
Fasting blood glucose (mmol/L)	-0.12 (7)	-0.14 (5)	-0.07 (2)	(-0.25, 0.01)	-0.3, 0.02)	(-0.31, 0.16)	0.07	0.09	0.54	0	2	0
Fasting insulin (mU/L)	0.38 (7)	0.28 (5)	0.47 (2)	(-0.74, 1.50)	(-1.34, 1.89)	(-1.08, 2.02)	0.51	0.74	0.55	0	0	0
HOMA index	0.08 (4)	0.05 (3)	0.10 (1)	(-0.32, 0.47)	(-0.51, 0.61)	(-0.46, 0.66)	0.71	0.86	0.73	0	0	0
Albumin (g/L)	0.58 (4)	-0.59 (2)	0.91 (2)	(-0.59, 1.74)	(-3.69, 2.51)	(-0.76, 2.58)	0.33	0.71	0.29	5	40	0
Muscle strength parameters												
Hand grip strength (pounds/inch^2)	0.23 (4)	0.47 (2)	-0.07 (2)	(-0.26, 0.73)	(--0.27, 1.20)	(-0.64, 0.50)	0.36	0.21	0.81	65	70	34
Knee extension strength (Nm/kg)	0.07 (6)	-0.10 (4)	0.28 (2)	(-0.26, 0.40)	(-0.47, 0.28)	(-0.25, 0.81)	0.68	0.62	0.30	48	24	61

H = healthy; I = inconsistency; S = sarcopenia; T = total; WMD = weighted mean difference.

amount of dietary protein in older participants. This might in part be explained by the fact that the present meta-analysis focused on studies with a running time of at least 10 days and on study participants aged at least 65 years. In consequence, most outcome parameters could be extracted from only a small number of trials potentially insufficient to yield significant results.

Increases in BW due to a supplementation with protein-energy formulas were reported for chronically ill and hospitalized persons aged 65 years or older in a meta-analysis by Milne and co-workers (50). Due to the condition of the study participants and to the fact that the systematic literature search was not restricted with respect to the kind of supplementation, a direct comparison with the present data is rather difficult. Changes in BW were accompanied by higher diameters in the musculature of the upper limbs in the study by Milne et al. (50), but it was not apparent whether this was the result of muscle mass, fat mass or elevated water content. As compared to controls, a significantly more pronounced gain in BW was observed in older persons taking a protein-rich supplement in a meta-analysis by Cawood et al. (48) as well. Some of the included trials were performed with healthy volunteers while others investigated the effects of protein supplementation on patients with hip fracture, diseases of the respiratory or the gastrointestinal tract, and kidney failure. The average age of the study participants was comparable to the present study (i.e. 72 years). LBM was assessed in the study by Cermak et al. (49). Following a combined intervention with protein supplements and resistance training, fat-free mass turned out to be significantly more augmented in the intervention group as compared to placebo. Subgroup analyses revealed that this effect was less pronounced in older volunteers (> 50 years) although it remained statistically significant.

In a meta-analysis by De Laet et al. (51), BMI was associated with a reduced risk of hip fracture. According to their findings, every additional BMI unit will lessen the fracture probability by 7% [RR: 0.93, 95%-CI (0.91, 0.94)], although in a non-linear fashion. Therefore, the fracture risk for individuals with a BMI of 20 kg/m^2 will be twice as high as for individuals with a BMI of 25 kg/m^2, [RR 1.95, 95%-CI (1.70, 2.22)], whereas another increase in BMI by 5 units (30 kg/m^2) will correspond to a 17% risk reduction only [RR 0.83, 95%-CI (0.69, 0.99)] (51). Thus, within the given BMI range found in the present meta-analysis, the reported step-up in BMI of 0.33 kg/m2 following leucine supplementation will most likely reflect only a small preventive measure. However, in order to become physiologically relevant, it is not mandatory for BW, BMI or LBM to be increased following a therapeutic intervention. For the elderly, it might be sufficient to stabilize temporary muscle mass. This is further emphasized by the findings of Brady et al. (52), who reported that lower muscle quality in overweight and obese females was not correlated with their respective BMI values.

A number of studies have pointed out the usability of LBM or related measures such as Lean Mass Index (LBM/m^2) as predictors of osteoporosis and fracture risk in post-menopausal women (53, 54). With manifested type 2 diabetes, LBM might be suitable as a prognostic marker for fracture incidents in a younger population of men and women as well (55). Moreover, in a longitudinal study by Lee et al. (56) performed at 6 clinical centres in the US and enrolling a total of 4.331 men aged 65 to 93 years, there was a correlation between a decline in LBM of more than 5% with respect to the initial values and all-cause mortality [HR=1.78 95%-CI (1.45, 2.19)]. The absolute values for loss of LBM within the population averaged 4 kg. Applied to the findings of the present meta-analysis, the mean restoration of LBM of 0.99 kg found after leucine supplementation would be equivalent to a decreased all-cause mortality risk of approximately 20%. In the same investigation (Lee et al., 2011), an inverse correlation was found between all-cause mortality and body weight [HR=1.84, 95%-CI (1.50, 2.26)]. A reduction in body weight of 5% with respect to the values at the beginning of the observation period would be equivalent to an absolute number of 6.5 kg. Thus, the average increase in body weight of 1.02 kg found in the present systematic review can be interpreted as a beneficial

consequence of leucine intervention as well.

None of the trials included in this meta-analysis reported any serious side-effects following supplementation with either leucine or leucine-rich formulas. In the study by Chale et al. (37), six of 80 volunteers complained about gastrointestinal discomfort, however, this could not be attributed to the supplement. All other particular incidents as well as reasons for drop-out (e.g. cardiovascular complications, infections, operations) were due to the general health conditions and the age of the study participants, but not due to any kind of leucine supplementation. To date, there is no defined No-Observed-Adverse-Effect-Level for leucine. A few studies investigated high-dose leucine or BCAA supplementation in healthy men. BCAA was tolerated without complications or side-effects in amounts of 14.4 g/d for 30 days (57), and in another study (58), leucine did not cause any acute health problems following applications up to 550 mg/kg BW (equal to approximately 40 g/d). Taken together, these high-dose administrations do not overlap with the lower amounts of leucine given in the trials included in the present meta-analysis. However, it should be noted that these were all short-term investigations. At least some of the anabolic effects of leucine are supposed to be mediated via the mammalian target of rapamycin complex 1 (mTORC1) known to be up-regulated in certain kinds of tumours (59). Following infusions with amino acids in patients with colorectal cancer, protein synthesis was increased both in skeletal muscle and in the tumour tissue (60). More studies are required investigating this potential long-time side-effect of amino acid supplementation, especially with respect to the safe amount of nutrient (efficient for stimulation of muscle protein synthesis without affecting other tissues). Another aspect of potential adverse effects might be related to the changes in anthropometrical data found in the present systematic review. Recently, Batsis and co-workers (61) observed reduced hazard ratios for cardiovascular mortality in older adults with elevated BMI (> 28.2. kg/m^2).

Strengths and limitations

The systematic literature search for this meta-analysis was last updated in February, 2014. Regarding publication bias, Funnel plots revealed only minor indications of small study effects. However, it cannot be excluded that unpublished data not considered in this analysis may have had at least a moderate impact on the effect size estimates. A major limitation often found in nutritional intervention studies is the heterogeneity of various aspects and characteristics of the study design. There were a large variety of supplementation protocols executed in the trials included in this meta-analysis (e.g. provision of leucine via whey or casein protein or given as a single supplement). Although it was possible to calculate the respective amount of leucine in each formula, it still remains possible that the changes in outcome parameters observed in these leucine groups are not due to this distinct amino acid but at least in part to other ingredients (essential amino acids, BCAA). In addition, synergistic effects between leucine and other amino acids or carbohydrates cannot be ruled out. It might be preferable for future studies to limit supplementation protocols to leucine alone in order to examine the isolated benefits of the nutrient on muscle protein synthesis. Moreover, not all studies enrolled in this systematic review reported on total energy consumption (Table 1A). An increase in energy intake during the course of the trial assessed in the supplemental group only, but not in controls, was reported by Bos et al. (36). Thus, it cannot be excluded that the effects observed in this meta-analysis are due to differences in caloric intake resulting from the interventions with leucine-rich formulas, i.e. an increase in total energy consumption might be a prerequisite for the success of these supplementations with respect to parameters such as LBM. Another limitation affecting the number of trials available for the present analysis was given by the study population. Participants were restricted with respect to age (≥ 65 years) and health status. In most interventions found during the literature search, individuals with sarcopenia were at the same time chronically ill or hospitalized due to post-operative or post-traumatic complications. Since these situations have a strong impact on protein metabolism, the corresponding studies were not included in the meta-analyses. On the other hand, the focus of this study on healthy or sarcopenic, but otherwise healthy subjects aged 65 or older can be interpreted as a specific strength of this systematic review as well. Finally, it should be noted that none of trials included in the present systematic review investigated on the potential biochemical mechanism of action by which leucine exerts its anabolic effects. Therefore, one can only speculate upon the involvement of mTORC1 (59) as well as other mediators.

Conclusion

In the present meta-analysis, leucine supplementation was found to exert beneficial effects on BW, BMI, and LBM in older persons, especially in those subjects already prone to sarcopenia. Therefore, these data support the current discussion for an evidence-based adaptation of the recommendations for dietary protein intake in the elderly (62). However, due to the large heterogeneity between studies included in this systematic review, dietary enrichment with leucine still remains controversial. Further studies are required adopting a more homogenous design with respect to participant characteristics, duration as well as the kind and daily amount of supplement used.

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Ethical standards: None

References

1. Evans WJ. What is sarcopenia? J Gerontol A Biol Sci Med Sci 50 Spec No:5-8, 1995.
2. Short KR, Vittone JL, Bigelow ML, Proctor DN, Nair KS. Age and aerobic exercise training effects on whole body and muscle protein metabolism. Am J Physiol Endocrinol Metab 2004;286:E92-101. doi: 10.1152/ajpendo.00366.2003
3. Wolfe RR. Regulation of muscle protein by amino acids. J Nutr 2002;132:3219S-3224S
4. Valerio A, D'Antona G, Nisoli E. Branched-chain amino acids, mitochondrial biogenesis, and healthspan: an evolutionary perspective. Aging (Albany NY) 2011;3:464-478
5. Frexes-Steed M, Lacy DB, Collins J, Abumrad NN. Role of leucine and other amino acids in regulating protein metabolism in vivo. Am J Physiol 1992;262:E925-935
6. Koopman R, Walrand S, Beelen M, Gijsen AP, Kies AK, Boirie Y, Saris WH, van Loon LJ. Dietary protein digestion and absorption rates and the subsequent postprandial muscle protein synthetic response do not differ between young and elderly men. J Nutr 2009;139:1707-1713. doi: 10.3945/jn.109.109173
7. Pennings B, Koopman R, Beelen M, Senden JM, Saris WH, van Loon LJ. Exercising before protein intake allows for greater use of dietary protein-derived amino acids for de novo muscle protein synthesis in both young and elderly men. Am J Clin Nutr 2011;93:322-331. doi: 10.3945/ajcn.2010.29649
8. Cuthbertson D, Smith K, Babraj J, Leese G, Waddell T, Atherton P, Wackerhage H, Taylor PM, Rennie MJ. Anabolic signaling deficits underlie amino acid resistance of wasting, aging muscle. FASEB J 2005;19:422-424. doi: 10.1096/fj.04-2640fje
9. Katsanos CS, Kobayashi H, Sheffield-Moore M, Aarsland A, Wolfe RR. Aging is associated with diminished accretion of muscle proteins after the ingestion of a small bolus of essential amino acids. Am J Clin Nutr 2005;82:1065-1073
10. van Loon LJ. Leucine as a pharmaconutrient in health and disease. Curr Opin Clin Nutr Metab Care 2012;15:71-77. doi: 10.1097/MCO.0b013e32834d617a
11. Leenders M, van Loon LJ. Leucine as a pharmaconutrient to prevent and treat sarcopenia and type 2 diabetes. Nutr Rev 2011;69:675-689. doi: 10.1111/j.1753-4887.2011.00443.x
12. Katsanos CS, Kobayashi H, Sheffield-Moore M, Aarsland A, Wolfe RR. A high proportion of leucine is required for optimal stimulation of the rate of muscle protein synthesis by essential amino acids in the elderly. Am J Physiol Endocrinol Metab 2006;291:E381-387. doi: 10.1152/ajpendo.00488.2005
13. Buse MG, Reid SS. Leucine. A possible regulator of protein turnover in muscle. J Clin Invest 1975;56:1250-1261. doi: 10.1172/JCI108201
14. Buse MG, Atwell R, Mancusi V. In vitro effect of branched chain amino acids on the ribosomal cycle in muscles of fasted rats. Horm Metab Res 1979;11:289-292
15. Garlick PJ, Grant I. Amino acid infusion increases the sensitivity of muscle protein synthesis in vivo to insulin. Effect of branched-chain amino acids. Biochem J 1979;254:579-584
16. Rieu I, Sornet C, Bayle G, Prugnaud J, Pouyet C, Balage M, Papet I, Grizard J, Dardevet D. Leucine-supplemented meal feeding for ten days beneficially affects postprandial muscle protein synthesis in old rats. J Nutr 2003;133:1198-1205
17. Bennet WM, Connacher AA, Scrimgeour CM, Smith K, Rennie MJ. Increase in anterior tibialis muscle protein synthesis in healthy man during mixed amino acid infusion: studies of incorporation of [1-13C]leucine. Clin Sci (Lond) 1989;76:447-454
18. Smith K, Reynolds N, Downie S, Patel A, Rennie MJ. Effects of flooding amino acids on incorporation of labeled amino acids into human muscle protein. Am J Physiol 1998;275:E73-78
19. Louard RJ, Barrett EJ, Gelfand RA. Effect of infused branched-chain amino acids on muscle and whole-body amino acid metabolism in man. Clin Sci (Lond) 1990;79:457-466
20. Nair KS, Schwartz RG, Welle S. Leucine as a regulator of whole body and skeletal muscle protein metabolism in humans. Am J Physiol 1992;263:E928-934
21. Paddon-Jones D, Sheffield-Moore M, Zhang XJ, Volpi E, Wolf SE, Aarsland A, Ferrando AA, Wolfe RR. Amino acid ingestion improves muscle protein synthesis in the young and elderly. Am J Physiol Endocrinol Metab 2004;286:E321-328. doi: 10.1152/ajpendo.00368.2003
22. Volpi E, Kobayashi H, Sheffield-Moore M, Mittendorfer B, Wolfe RR. Essential amino acids are primarily responsible for the amino acid stimulation of muscle protein anabolism in healthy elderly adults. Am J Clin Nutr 2003;78:250-258
23. Koopman R, Verdijk L, Manders RJ, Gijsen AP, Gorselink M, Pijpers E, Wagenmakers AJ, van Loon LJ. Co-ingestion of protein and leucine stimulates muscle protein synthesis rates to the same extent in young and elderly lean men. Am J Clin Nutr 2006;84:623-632
24. Børsheim E, Bui QU, Tissier S, Kobayashi H, Ferrando AA, Wolfe RR. Effect of amino acid supplementation on muscle mass, strength and physical function in elderly. Clin Nutr 2008;27:189-195. doi: 10.1016/j.clnu.2008.01.001
25. Verhoeven S, Vanschoonbeek K, Verdijk LB, Koopman R, Wodzig WK, Dendale P, van Loon LJ. Long-term leucine supplementation does not increase muscle mass or strength in healthy elderly men. Am J Clin Nutr 2009;89:1468-1475. doi: 10.3945/ajcn.2008.26668
26. Moher D, Liberati A, Tetzlaff J, Altman DG, Group P. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. PLoS Med 2009;6:e1000097. doi: 10.1371/journal.pmed.1000097
27. Yang Y, Breen L, Burd NA, Hector AJ, Churchward-Venne TA, Josse AR, Tarnopolsky MA, Phillips SM. Resistance exercise enhances myofibrillar protein synthesis with graded intakes of whey protein in older men. Br J Nutr 2012;108:1780-1788. doi: 10.1017/S0007114511007422
28. Higgins JP, Altman DG, Gøtzsche PC, Jüni P, Moher D, Oxman AD, Savovic J, Schulz KF, Weeks L, Sterne JA, Group CBM, Group CSM. The Cochrane Collaboration's tool for assessing risk of bias in randomised trials. BMJ 2011;343:d5928
29. Hooper L, Summerbell CD, Thompson R, Sills D, Roberts FG, Moore HJ, Davey Smith G. Reduced or modified dietary fat for preventing cardiovascular disease. Cochrane Database Syst Rev 2012;5:CD002137. doi: 10.1002/14651858.CD002137.pub3
30. Higgins JP, Green S (Version 5.1.0 updated March 2011) Cochrane Handbook of systematic reviews. In.
31. Higgins JP, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. BMJ 2003;327:557-560. doi: 10.1136/bmj.327.7414.557
32. Björkman M, Finne-Soveri H, Tilvis RS (2012) Whey protein supplementation in nursing home residents. A randomized controlled trial. European Geritatric Medicine 2012;3:161-166
33. Hozo SP, Djulbegovic B, Hozo I. Estimating the mean and variance from the median, range, and the size of a sample. BMC Med Res Methodol 2005;5:13. doi: 10.1186/1471-2288-5-13
34. Alemán-Mateo H, Macías L, Esparza-Romero J, Astiazaran-García H, Blancas AL. Physiological effects beyond the significant gain in muscle mass in sarcopenic elderly men: evidence from a randomized clinical trial using a protein-rich food. Clin Interv Aging 2012;7:225-234. doi: 10.2147/CIA.S32356
35. Bonnefoy M, Laville M, Ecochard R, Jusot JF, Normand S, Maillot S, Lebreton B, Jauffret M. Effects of branched amino acids supplementation in malnourished elderly with catabolic status. J Nutr Health Aging 2010;14:579-584
36. Bos C, Benamouzig R, Bruhat A, Roux C, Valensi P, Ferrière F, Tomé D. Nutritional status after short-term dietary supplementation in hospitalized malnourished geriatric patients. Clin Nutr 2001;20:225-233. doi: 10.1054/clnu.2000.0387
37. Chalé A, Cloutier GJ, Hau C, Phillips EM, Dallal GE, Fielding RA. Efficacy of whey protein supplementation on resistance exercise-induced changes in lean mass, muscle strength, and physical function in mobility-limited older adults. J Gerontol A Biol Sci Med Sci 2013;68:682-690. doi: 10.1093/gerona/gls221
38. Dillon EL, Sheffield-Moore M, Paddon-Jones D, Gilkison C, Sanford AP, Casperson SL, Jiang J, Chinkes DL, Urban RJ. Amino acid supplementation increases lean body mass, basal muscle protein synthesis, and insulin-like growth factor-I expression in older women. J Clin Endocrinol Metab 2009;94:1630-1637. doi: 10.1210/jc.2008-1564
39. Godard MP, Williamson DL, Trappe SW. Oral amino-acid provision does not affect muscle strength or size gains in older men. Med Sci Sports Exerc 2002;34:1126-1131
40. Hays NP, Kim H, Wells AM, Kajkenova O, Evans WJ. Effects of whey and fortified collagen hydrolysate protein supplements on nitrogen balance and body composition in older women. J Am Diet Assoc 2009;109:1082-1087. doi: 10.1016/j.jada.2009.03.003
41. Hodgson JM, Zhu K, Lewis JR, Kerr D, Meng X, Solah V, Devine A, Binns CW, Woodman RJ, Prince RL. Long-term effects of a protein-enriched diet on blood pressure in older women. Br J Nutr 2012;107:1664-1672. doi: 10.1017/S0007114511004740
42. Kim HK, Suzuki T, Saito K, Yoshida H, Kobayashi H, Kato H, Katayama M. Effects of exercise and amino acid supplementation on body composition and physical function in community-dwelling elderly Japanese sarcopenic women: a randomized controlled trial. J Am Geriatr Soc 2012;60:16-23. doi: 10.1111/j.1532-5415.2011.03776.x
43. Kim CO, Lee KR. Preventive effect of protein-energy supplementation on the functional decline of frail older adults with low socioeconomic status: a community-based randomized controlled study. J Gerontol A Biol Sci Med Sci 2013;68:309-316. doi: 10.1093/gerona/gls167
44. Leenders M, Verdijk LB, van der Hoeven L, van Kranenburg J, Hartgens F, Wodzig WK, Saris WH, van Loon LJ. Prolonged leucine supplementation does not augment muscle mass or affect glycemic control in elderly type 2 diabetic men. J Nutr 2011;141:1070-1076. doi: 10.3945/jn.111.138495
45. Rondanelli M, Opizzi A, Antoniello N, Boschi F, Iadarola P, Pasini E, Aquilani R, Dioguardi FS. Effect of essential amino acid supplementation on quality of life, amino acid profile and strength in institutionalized elderly patients. Clin Nutr 2011;30:571-577. doi: 10.1016/j.clnu.2011.04.005
46. Scognamiglio R, Negut C, Piccolotto R, Dioguardi FS, Tiengo A, Avogaro A. Effects of oral amino acid supplementation on myocardial function in patients with type 2 diabetes mellitus. Am Heart J 2004;147:1106-1112. doi: 10.1016/j.ahj.2003.12.003
47. Tieland M, van de Rest O, Dirks ML, van der Zwaluw N, Mensink M, van Loon LJ, de Groot LC. Protein supplementation improves physical performance in frail elderly people: a randomized, double-blind, placebo-controlled trial. J Am Med Dir Assoc

2012;13:720-726. doi: 10.1016/j.jamda.2012.07.005
48. Cawood AL, Elia M, Stratton RJ. Systematic review and meta-analysis of the effects of high protein oral nutritional supplements. Ageing Res Rev 2012;11:278-296. doi: 10.1016/j.arr.2011.12.008
49. Cermak NM, Res PT, de Groot LC, Saris WH, van Loon LJ. Protein supplementation augments the adaptive response of skeletal muscle to resistance-type exercise training: a meta-analysis. Am J Clin Nutr 2012;96:1454-1464. doi: 10.3945/ajcn.112.037556
50. Milne AC, Avenell A, Potter J. Meta-analysis: protein and energy supplementation in older people. Ann Intern Med 2006;144:37-48
51. De Laet C, Kanis JA, Odén A, Johanson H, Johnell O, Delmas P, Eisman JA, Kroger H, Fujiwara S, Garnero P, McCloskey EV, Mellstrom D, Melton LJ, Meunier PJ, Pols HA, Reeve J, Silman A, Tenenhouse A. Body mass index as a predictor of fracture risk: a meta-analysis. Osteoporos Int 2005;16:1330-1338. doi: 10.1007/s00198-005-1863-y
52. Brady AO, Straight CR, Schmidt MD, Evans EM. Impact of body mass index on the relationship between muslce qualioty and physical function in older women. J Nutr Health Aging 2014;18: 378-382. doi: 10.1007/s12603-013-0421-0
53. Rikkonen T, Sirola J, Salovaara K, Tuppurainen M, Jurvelin JS, Honkanen R, Kröger H. Muscle strength and body composition are clinical indicators of osteoporosis. Calcif Tissue Int 2012;91:131-138. doi: 10.1007/s00223-012-9618-1
54. Zhang Z, Shen X, Zhang H, Li S, Zhou H, Wu X, Sheng Z, Liao E. The relationship between body composition and fracture risk using the FRAX model in central south Chinese postmenopausal women. Clin Endocrinol (Oxf) 2012;77:524-530. doi: 10.1111/j.1365-2265.2012.04399.x
55. Moseley KF, Dobrosielski DA, Stewart KJ, Sellmeyer DE, Jan De Beur SM. Lean mass predicts hip geometry in men and women with non-insulin-requiring type 2 diabetes mellitus. J Clin Densitom 2011;14:332-339. doi: 10.1016/j.jocd.2011.04.007
56. Lee CG, Boyko EJ, Nielson CM, Stefanick ML, Bauer DC, Hoffman AR, Dam TT, Lapidus JA, Cawthon PM, Ensrud KE, Orwoll ES, Group OFiMS. Mortality risk in older men associated with changes in weight, lean mass, and fat mass. J Am Geriatr Soc 2011;59:233-240. doi: 10.1111/j.1532-5415.2010.03245.x
57. De Lorenzo A, Petroni ML, Masala S, Melchiorri G, Pietrantuono M, Perriello G, Andreoli A. Effect of acute and chronic branched-chain amino acids on energy metabolism and muscle performance. Diabetes Nutr Metab 2003;16:291-297
58. Pencharz PB, Elango R, Ball RO. Determination of the tolerable upper intake level of leucine in adult men. J Nutr 2012;142:2220S-2224S. doi: 10.3945/jn.112.160259
59. Laplante M, Sabatini DM. mTOR signaling in growth control and disease. Cell 2012;149:274-293. doi: 10.1016/j.cell.2012.03.017
60. McNurlan MA, Heys SD, Park KG, Broom J, Brown DS, Eremin O, Garlick PJ. Tumour and host tissue responses to branched-chain amino acid supplementation of patients with cancer. Clin Sci (Lond) 1994;86:339-345
61. Batsis JA, Singh S, Lopez-Jimenez F. Anthropometric measurements and survival in older americans: results from the third National Health And Nutrition Examination Survey. J Nutr Health Aging 2014;18:123-130. doi: 10.1007/s12603-013-0366-3
62. Bauer J, Biolo G, Cederholm T, Cesari M, Cruz-Jentoft AJ, Morley JE, Phillips S, Sieber C, Stehle P, Teta D, Visvanathan R, Volpi E, Boirie Y. Evidence-based recommendations for optimal dietary protein intake in older people: a position paper from the PROT-AGE Study Group. J Am Med Dir Assoc 2013;14:542-559. doi: 10.1016/j.jamda.2013.05.021