Systematic review and meta-analysis of the effect of protein and amino acid supplements in older adults with acute or chronic conditions

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Abstract

The loss of lean body mass, muscle strength and physical function causes significant problems in older adults. Protein and amino acid supplements can preserve muscle strength but the effect on function is variable. We conducted a systematic literature review and meta-analysis to investigate the effect of protein and amino acid supplementation on fat-free mass, muscle strength and physical function in malnourished, frail, sarcopenic, dependent or elderly with acute or chronic conditions, with or without rehabilitation exercise. Databases searched included Medline, BIOSIS, CINAHL, Cochrane Library, EBM Reviews, Embase, Pre-Medline, ProQuest, PubMed and Scopus. Retrieved articles were assessed by two reviewers using the Cochrane Risk of Bias (ROB) Tool. In all, thirty nine randomised controlled trails (*n* 4274) were included. The studies used a range of protein or essential amino acid (EAA) supplements in a variety of settings, including hospital, community and long-term care. Only seven studies had low ROB and no effect of supplementation was found on any outcomes. Analysis of all thirty-nine studies suggest protein and EAA supplements may improve fat-free mass, muscle strength and physical function (standardised mean difference 0·21–0·27, all *P* < 0·005), but significant heterogeneity and ROB was evident. Predetermined subgroup analysis found undernourished elderly benefitted most; EAA were the most effective supplements and small beneficial effects were seen without rehabilitation exercise. The high heterogeneity and few studies with low ROB limits the conclusions and more high quality studies are needed to determine the best nutritional strategies for the maintenance of strength and function with increasing age.

Key words: Dietary proteins: Sarcopenia: Physical function: Muscle strength

Advancing age is accompanied by progressive decline in skeletal muscle mass, leading to the development of sarcopenia[1], which results in significant disability, mortality and health care costs[2,3]. Sarcopenia affects 5–13 % of 60–70 year olds and up to 50 % in those aged more than 80 years with higher prevalence in elderly with disease conditions[4]. The world's ageing population is expected to reach 1·5 billion in 2050[5] and due to the increased use of health and aged care resources, it is important to determine ways to maintain and improve the health of elderly people before muscle deterioration worsens life outcomes.

Preservation of muscle mass and stimulation of muscle protein synthesis (MPS) through nutritional intervention is a potential therapy for sarcopenia[6,7]. MPS is stimulated by protein and amino acid (AA) intake[6,8–10]. In older adults, inadequate dietary intake of protein[7,10] and the decreased anabolic response to dietary protein intake are believed to contribute to the loss of muscle mass[8,11]. Research has further indicated that essential

amino acids (EAA) are key regulators of MPS, with leucine a potent muscle stimulator[12,13] and may suppress muscle protein breakdown[10]. EAA supplements containing leucine have produced improvements in physical function in some studies[14–16]. Dosage and timing of protein intake may also contribute, with studies[17,18] demonstrating that protein consumed in ≥30 g bolus intakes are more effective in increasing post-prandial plasma AA concentration, a signal that stimulates MPS[18], and may have the additive effect to overcome the anabolic threshold to MPS[17].

Recent reviews have shown supplements, including high protein oral nutritional supplement (ONS)[19], EAA[20] or higher protein diets[21] improve muscle strength in older adults, but these reviews included healthy and frail elderly participants and included studies using resistance training (RT)[19–22]. Although RT is effective in improving muscle strength, it may not be viable for all patients. Nutritional supplementation alone or in association with geriatric rehabilitation may be a practical

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method for improving muscle mass and function, particularly in vulnerable participants such as those that are hospitalised, malnourished or sarcopenic and who are unwilling or unable to undertake RT.

The aim of this meta-analysis, therefore, is to identify evidence for the effectiveness of protein supplements on muscle mass, strength and physical function in malnourished, frail, sarcopenic, dependent or elderly with acute or chronic conditions, alone or in association with rehabilitation, but not undertaking RT and mirrors usual care provided for the elderly.

Methods

This meta-analysis used the Preferred Reporting Items for Systematic Reviews and Meta-Analyses Guidelines[23] (online Supplmentary Table S1). A literature search was conducted in April 2014 and updated on 17 June 2016, in the databases Medline, BIOSIS, CINAHL, Cochrane Library, EBM Reviews, Embase, Pre-Medline, ProQuest, PubMed and Scopus. Search terms used were synonyms and combinations of: dietary protein, whey, leucine, AA, nutritional status, nutrition supplement, skeletal muscle, muscle weakness, muscle atrophy, muscle strength, muscle function, sarcopenia, cachexia, weight changes, body composition, malnutrition, frailty, elderly and ageing. The search strategy appears in the online Supplementary Table S2. Citations were downloaded into Endnote X7. Interventions retrieved involved participants with mean age ≥65 years and enrolled volunteers aged 60 years or older, and compared increasing protein intake through protein-rich sources of food; food fortification; and orally consumed liquid, powder or tablets containing protein and energy or EAA, as EAA mixtures or single EAA, for example, leucine to a placebo; supplement of lower protein and energy content; usual care; or no dietary intervention control group. Studies of dietary advice alone were not included. We included studies enrolling hospitalised, community-dwelling or institutionalised subjects with conditions such as frailty, malnutrition, sarcopenia, post-orthopaedic surgery, fracture or acute disease. Studies focusing solely on RT, enteral or parenteral nutrition, or with young, well or healthy adults, or older adults with cancer, cirrhosis, renal disease, stroke or gastrointestinal surgery were excluded. Studies with aerobic or physical rehabilitation activities, without use of RT (defined as 60 % of one-repetition maximum)[24], were included only if identical rehabilitation components were provided to both intervention and control groups.

Titles and abstracts were checked for relevance, and coded for retrieval or exclusion. Reference lists of included studies and systematic literature reviews were checked for additional articles. Retrieved articles were coded and assessed by two reviewers using the Cochrane Risk of Bias (ROB) Tool[25]. Study data from eligible studies were extracted and tabulated for participant characteristics, numbers and withdrawals; type, dose, frequency of intervention; compliance to intervention; and outcome measures for fat-free mass (FFM), muscle strength, and physical function. In order to ascertain the most effective interventions in well-designed studies, studies with low protein density ONS (10 to <15 % energy from protein) or high ROB were excluded from the analysis[26–39].

Data extraction

Results for FFM, muscle strength, and physical function were extracted and analysed separately, either as changes from baseline, or baseline and endpoint scores. If the mean and standard deviation were not provided, the median was used and the SD estimated from the interquartile range. Sensitivity analyses indicated that leaving out one study at a time, did not change overall results appreciably. A correlation of 0·75 was used when variance data of the mean difference were not available[40]. Sensitivity analyses was undertaken and showed that the exact correlation value used made little difference to the overall results. Where data was reported without a quantitative value, that is in graphs or figures, study authors were contacted for further information. More than one outcome measure was commonly provided for the outcome of physical function; however, only one measure was included. The outcome measure chosen for inclusion was based on the characteristics of the instrument, that is tools that measured rather than collected self-reported information, and tools testing more advanced functional outcomes, for example walking speed was selected over Katz Activities of Daily Living (ADL). Tools administered more frequently in studies were used in an effort to reduce variability; for example, tests of walking speed were assessed in seven studies[33,41–46] and was the measure selected where data was available. If data for multiple time-points were available, the data closest to intervention end were used, as this was the most common time-point analysed.

Subgroup analysis

Pre-determined subgroup analyses were performed based on ROB score, participant characteristics, protein intervention type, and if participants undertook aerobic or physical rehabilitation activities. Participant characteristics were grouped by trial setting (hospital, long-term care (LTC) in institutions, or community) and nutritional status (nourished or undernourished) as defined by study authors, or with low body weight or BMI <25 kg/m² as used in a previous review[47]. Interventions were characterised as protein-rich foods; EAA (as EAA mixtures or single types of EAA); ONS with protein density of 10 to <15 % (low), 15 to ≤20 % (moderate) or >20 % (high) composition of total energy; and whey-based ONS. Regression based on study duration was also undertaken.

Post hoc analyses was undertaken to determine potential effects of additional nutrients (intrinsically in protein-rich foods, or added as a supplement, e.g. in ONS) and differential effects in studies using a placebo, with meta-regression based on grams of protein or EAA provided by the intervention to determine the effect of dose.

Statistical analysis

Meta-analysis was performed using Comprehensive Meta-Analysis version 2 (Biostat) using the random effects model for heterogeneous samples, as participants were of various health statuses and heterogeneity was likely to be high. Heterogeneity was analysed using the I^2 statistic[48], with

P values used to identify statistically significant heterogeneity. No significant heterogeneity was found for the FFM analysis; however, the random effects model was used across the three outcome variables for consistency. Effect size was reported as the standardised mean difference (SMD) and the 95% CI. General rules of thumb for effect size used were 0·2 for 'small', 0·5 for 'moderate' and 0·8 for 'large' effect[49]. Overall significance was assumed at $P < 0.05$. Meta-regression on additional EAA and protein dose provided through interventions and intervention length were undertaken and presented as slope, standard error. Meta-analysis data were presented as forest plots, and publication bias was assessed with funnel plots.

Results

A total of 8702 citations were reviewed, with thirty-nine studies fulfilling the selection criteria (Fig. 1).
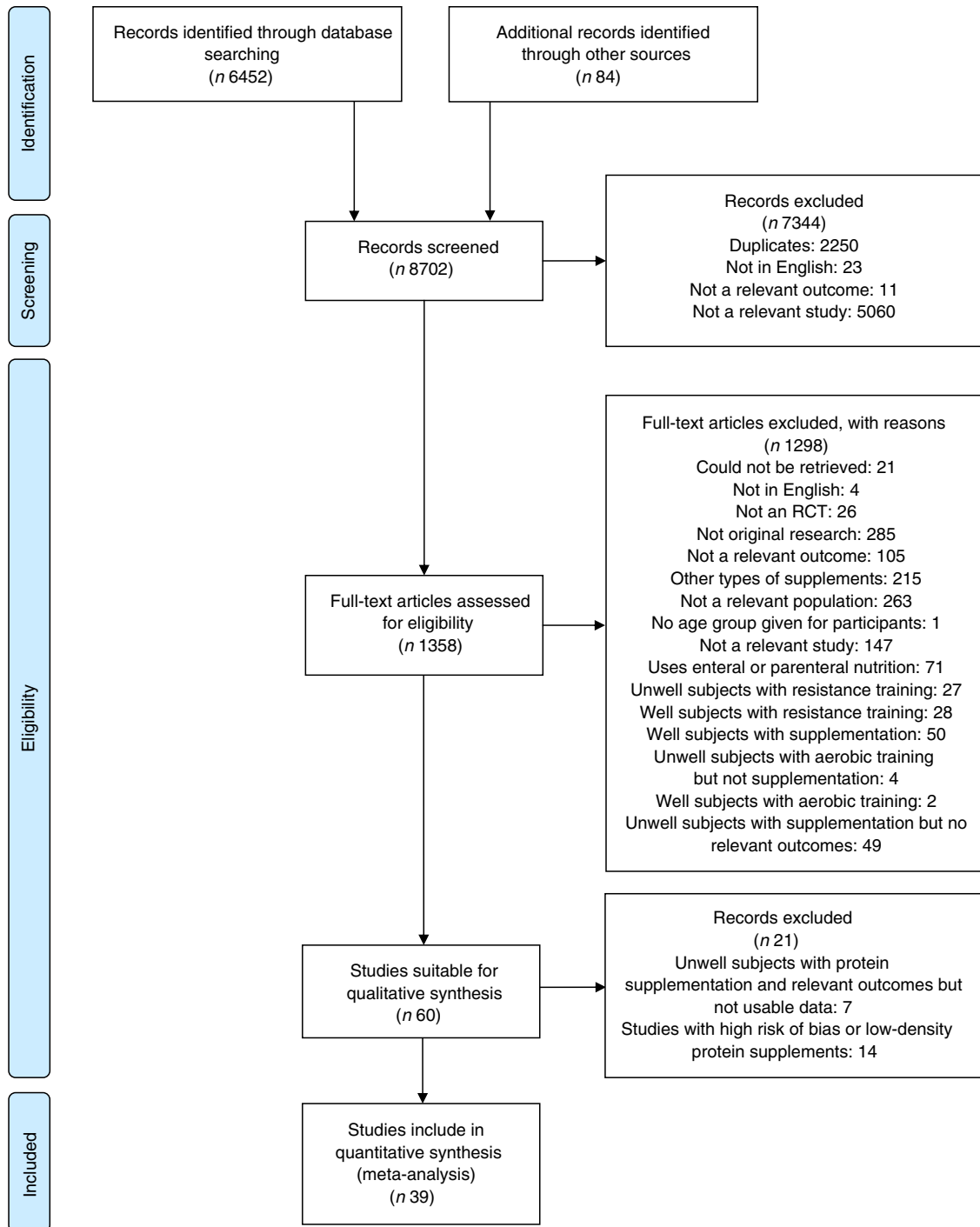

Fig. 1. Preferred Reporting Items for Systematic Reviews and Meta-Analyses flow diagram summarising selection process. RCT, randomised controlled trails.

Publication bias and sensitivity analysis

No obvious evidence of publication bias was found for studies of FFM and physical function, with only minor evidence of publication bias for studies of muscle function, as indicated by the funnel plots (see online Supplementary Fig. S1(a)–(c)). Sensitivity analysis showed small changes in SMD after removal of one study in each outcome analysis, but *P* values remained significant (all *P* < 0·01), and all thirty-nine studies were included in the analysis. The weights of individual studies ranged from 2·18 to 12·51 for FFM, 2·05 to 5·10 for muscle strength, and 1·02 to 5·48 for physical function.

Characteristics of included studies

A summary of the main characteristics of the thirty-nine studies that met inclusion criteria can be found in Table 1. Two authors who were contacted provided additional information not reported in the original studies[50,51]. The included studies involved 4274 participants, with ages ranging from 60[43,52] to 103[53]. In all, sixteen studies involved community-dwelling participants (*n* 2350); twelve in hospital (*n* 1264); and eleven in LTC settings, including residential care and nursing homes (*n* 660). Most of the studies included undernourished, at risk of malnutrition, sarcopenic or frail participants, with thirteen studies including elderly participants with a mean normal weight:height ratio (BMI) ≥25 kg/m².

Thirty-one studies involved supplementation with ONS and protein-rich foods[41,43,44,46,50,51,53–77], three of which used whey protein-based ONS[56,57,73]. Eight studies used EAA supplementation, either as EAA mixtures with or without other nutrients[14,16,45,52,78,79] or leucine supplementation only[80]. Study duration ranged from 2 weeks[77] to 24 months[62], with twenty-six to 1006 participants included. The health characteristics of participants varied, with ten studies focusing on frail participants (*n* 781); three studies on sarcopenic participants (*n* 488); and fifteen studies with participants who were malnourished, undernourished or at risk of malnutrition (*n* 2066). The remaining studies included participants diagnosed with, or recovering from infections or acute illness (*n* 550); fractures or other surgical operations (*n* 540); wound healing difficulties (*n* 38); and catabolic diseases (*n* 30). Nine studies involved participants with long-term conditions, such as including coronary artery disease, chronic heart failure, type 2 diabetes mellitus, chronic obstructive pulmonary disease, osteoporosis, the metabolic syndrome and dementia (*n* 517).

Of the thirty-one studies with ONS and protein-rich food interventions, sixteen studies used attention control, no dietary intervention, or standard dietetic care as a comparator; eleven studies used a protein-free comparator; one used a protein-free placebo; and three used alternative ONS with lower energy and protein content. Interventions in these studies could increase energy and protein intake by 355 kJ (85 kcal)[71] to 5040 kJ (1200 kcal)[68], and 4[71] to 50 g[64], respectively. Mean compliance for ONS intake varied from 54 to 100 %.

Of the eight studies using EAA supplementation, five studies used placebos as comparators, and the remaining studies used attention control, no dietary intervention or standard dietetic care. EAA and leucine intake could increase by 3[79] to 40 g/d[52], and 1·2[79] to 7·5 g/d[80], respectively. Only three studies reported compliance to supplements and comparators, of 72 to 100 and 72 %, respectively.

A total of twelve studies involved aerobic or physical rehabilitation activities, which included use of activities as part of standard care[52,59,69,75]; participants recruited from rehabilitation units[50]; participants discharged from hospital to rehabilitation facilities[67,68,72]; and as novel health interventions[43,57,73,76].

Risk of bias assessment

Seven studies were at low and thirty-two at unclear ROB. Intention to treat analysis was carried out in eleven studies. Randomisation was inadequately reported in nine studies[53,55,57,60,66,71,75–77]. Participants were blinded in only nineteen studies[14,16,43,44,46,52,56,58,61,63,65,67,70,72–74,78,80,81], mainly due to lack of use of a placebo; researchers were blinded in eighteen studies[14–16,41,45,46,52,54,56,61,63,69,72–74,78,80,81] and outcome assessors in fifteen studies[41,43,45,46,51,56,59,61,62,67–70,74].

Outcomes

Forest plots from the analysis of FFM, muscle strength and physical function outcomes can be found in Figs 2–4, respectively. Statistics, including heterogeneity for all subgroup analyses are reported in the online Supplementary Table S3 with significant effect size results reported below. Studies were alphabetically coded 'a' or 'b' for studies with multiple intervention arms[55,79] or if outcome reporting was separated by intervention subgroups[75].

Body composition

In all, sixteen studies reported data on FFM (analysed *n* 970) with methods including dual-energy X-ray absorptiometry and bioelectrical impedance analysis. The effect of ONS and EAA supplementation on FFM was significant with a small effect size (SMD 0·21; 95 % CI 0·07, 0·35, *P* < 0·01, no evidence of heterogeneity, $I^2 = 15·1$, *P* = 0·28) (Fig. 2). Only one study had low ROB[74], with no significant effect on FFM seen. However, a small effect was seen in studies with unclear ROB (SMD 0·23; 95 % CI 0·08, 0·37, *P* < 0·01, no evidence of heterogeneity, $I^2 = 17·6$, *P* = 0·25). Other subgroup analysis showed improvements in FFM in studies using protein-rich foods (SMD 0·26; 95 % CI 0·02, 0·50, *P* = 0·03, no evidence of heterogeneity, $I^2 = 0·0$, *P* = 0·43); studies using supplements with additional vitamins or minerals (SMD 0·24; 95 % CI 0·09, 0·38, *P* < 0·01, no evidence of heterogeneity, $I^2 = 0·0$, *P* = 0·56) and with patients not participating in rehabilitation programmes (SMD 0·23; 95 % CI 0·06, 0·41, *P* = 0·01, no evidence of heterogeneity, $I^2 = 29·3$, *P* = 0·14) (online Supplementary Table S3). Meta-regression of the protein (slope 0·007, SE 0·012, *P* = 0·57) or EAA (slope 0·08, SE 0·15, *P* = 0·60) dose provided in interventions showed no evidence of dose–response, although the number of EAA studies was small (*n* 4). Duration of intervention also showed no significant effect (slope −0·003, SE 0·007, *P* = 0·62). Studies (*n* 4) using a true placebo showed no significant effect.

Table 1. Characteristics of included studies

First author, year	Study ROB	Population sample size (IG/CG)	Mean age (years), baseline BMI (kg/m^2)	Study length	Ingestion details	Dose. Total daily intervention protein and additional nutrients	Rehab (Y/N)	Comparator/control
Abe, 2016	U	Frail NH residents a: MCT:13/12 b: LCT: 13/12	MCT IG: 85·5, BMI 18·7 LCT IG: 87·7, BMI 18·8 CG: 86·8, BMI 19·6	3 months	1/d, at D	a: MCT IG: 6 g MCT, 3·0 g EAA, 20 μg vit. D. 334 kJ, 1·2 g Leu/d. b: LCT IG: 6 g LCT, 3·0 g EAA, 20 μg vit. D. 351 kJ, 1·2 g Leu/d	N	No treatment
Aleman-Mateo, 2012	U	Community sarcopenic 20/20	IG: 75·4, BMI 26·5 CG: 76·7, BMI 26·1	3 months	3/d, at BF, L, D	70 g ricotta*. 1116 kJ, 15·7 g P/d	N	Usual diet and home visits
Aquiliani, 2008	U	Outpatients, CHF 22/22	IG: 74·5, BMI 22·5 CG: 73·1, BMI 23·2	2 months	2/d, at morning, afternoon	4 g EAA. 300 kJ, 8 g EAA, 2·5 g Leu/d	N	No treatment
Bakhitiari, 2012	U	Community a: Soya: 25/25 b: TSP 25/25	Soya IG: 63·8, BMI 28·8 TSP IG: 64·6, BMI 27·5 CG: 64·1, BMI 28·5	12 weeks	1/d	a: Soya IG: 35 g soya nut*. 750 kJ, 13·8 g P/d b: TSP IG: 35 g TSP*. 512 kJ, 18·2 g P/d	N	No treatment
Bauer, 2015	L	Sarcopenic 184/196	IG: 77·3, BMI 26·0 CG: 78·1, BMI 26·2	13 w	2/d, before BF, L (IG and CG)	20 g Leu-enriched whey protein in water, 20 μg vit. D. 1254 kJ, 41·4 g P, 6 g Leu/d	N	Placebo. 1254 kJ, 0 g P/d
Björkman, 2012	U	NH residents 49/57	IG: 84·1, BMI 24·8 CG: 83·0, BMI 24·0	6 months	3/d (IG and CG)	150 ml juice with whey protein*. 1181 kJ, 20 g P/d	Y	150 ml juice*. 846 kJ, 0 g P/d
Bonnefoy, 2003	U	Retirement home frail 30/27	IG: 83, BMI 27·13 CG: 83, BMI 27·32	9 months	2/d, at morning, afternoon (IG and CG)	200 ml ONS*. 1686 kJ, 30 g P/d	N	0 kJ placebo
Bonnefoy, 2010	U	Malnourished catabolic 15/15	IG: 82·5, BMI 22·6 CG: 79·4, BMI 23·4	2 weeks	3–5/d, at L, D	6·5–9 g P (47·5 % BCAA). 213–357 kJ, 11·0–18·0 g P, 3·6–6·0 g Leu/d. Added to food	N	Usual dietetic advice
Bouillanne, 2013	U	Rehabilitation Malnourished/at risk 30/36	IG: 84·1, BMI 20·7 CG: 85·7, BMI 20·9	6 weeks	72 % P intake at L	Protein-rich food and 6 g milk protein powder. To achieve 1·5 g P/kg BW/d intake	Y	Usual diet, even spread kJ and P
Cameron, 2011	U	Undernourished fracture 23/21	IG: 83·7, BMI 21·5 CG: 87·1, BMI 21·5	40 d	1/d (IG and CG)	Approximately 235 ml milk- or milk and soya-based ONS*. 1475–1986 kJ, 17·6–21·3 g P/d	Y	HP diet with HP milk. 811 kJ, 11g P/d
Chapman, 2009	U	Community Undernourished 13/13	IG: 78·5, BMI 18·7 CG: 78·5, BMI 19	12 months	1/d, after meal	237 ml milk-based ONS*. 1986 kJ, 21·6 g P/d	N	Usual care
Collins, 2005	U	NH, wound clinic 18/20	IG: 79·2, BMI 25·5 CG: 81·0, BMI 26·4	4 weeks	3/d, with meals (IG and CG)	80 ml, 8·4 kJ (2 kcal)/ml ONS*. 1995 kJ, 19·8 g P/d	N	80 ml, 4·2 kJ (1 kcal)/ml ONS*. 1050 kJ, 8·8 g P/d
Dal Negro, 2010	U	Outpatients sarcopenic COPD 16/16	IG: 75, BMI 20·2 CG: 75, BMI 20·2	12 weeks	2/d (IG and CG)	4 g EAA. 8 g EAA/d	N	Placebo
Dal Negro, 2012	U	Outpatients sarcopenic COPD 44/44	IG: 75, BMI 20·0 CG: 73, BMI 20·1	12 weeks	2/d, at morning and afternoon (IG and CG)	4 g EAA. 183 kJ, 8 g EAA, 2·5 g Leu/d	N	Placebo
Dangour, 2011	U	Community Low-middle SES 502/504	All participants 65–67·9 IG: BMI 28·2 CG: BMI 28·6	24 months	1/d	50 g vegetable powder* and 50 g low lactose milk powder*. 1685 kJ, 15·5 g P/d	N	No treatment
Dreyer, 2013	U	Post-surgical, TKA 21/19	IG: 68, BMI 34 CG: 70, BMI 29	3 weeks	2/d, at morning and afternoon (IG and CG)	20 g EAA. 7·2 g Leu/d	Y	2/d, 20 g alanine
Espaulella, 2000	L	Inpatients, fracture 85/86	IG: 82·4, BMI 25·4 CG: 82·7, BMI 25·4	60 d + 6 months F/U	1/d (IG and CG)	200 ml ONS*/d. 623 kJ, 20 g P/d	N	200 ml placebo. 648 kJ, 0 g P/d
Fiatarone, 1994	U	NH, frail 24/26	IG: 85·7, BMI 25·4 CG: 89·2, BMI 25·8	10 weeks	1/d, at PM (IG and CG)	240 ml soya-based ONS*. 1505 kJ, 15·3 g P/d	N	240 ml placebo. 17 kJ/d
Gariballa, 2007	L	Hospital 106/119	IG: 75·9, BMI 25·2 CG: 75·3, BMI 25·2	6 weeks +6 months F/U	2/d, at morning and afternoon (IG and CG)	200 ml ONS*. 4160 kJ, 49·8 g P/d	N	251 kJ placebo
Kim, 2012	U	Community sarcopenic 39/39	IG: 79·2, BMI 18·9 CG: 78·7, BMI 18·8	3 months	2/d, in milk/water	3 g EAA. 6 g EAA, 2·5 g Leu/d	N	Health education classes
Kim, 2013	U	Community, frail 43/44	IG: 78·9 CG: 78·4	12 weeks	2/d	200 ml ONS*. 1672 kJ, 25 g P/d	N	Home visits
Lauque, 2000	U	NH, malnourished 19/22	IG: 84·6, BMI 22·3 CG: 84·7, BMI 21·8	60 d	1–2/d, with meals	150–200 ml milk-, whey- or milk and pea protein-based ONS*. 1254–2090 kJ, 24–39 g P/d	N	No treatment

Table 1. *Continued*

First author, year	Study ROB	Population sample size (IG/CG)	Mean age (years), baseline BMI (kg/m²)	Study length	Ingestion details	Dose. Total daily intervention protein and additional nutrients	Rehab (Y/N)	Comparator/control
Lauque, 2004	U	Hospital, AD care 46/45	IG: 79·5, BMI 22·2 CG: 78·1, BMI 22·6	3 months +3 months F/U	1–2/d	150–200 ml milk- or milk and pea protein-based ONS*. 1254–2090 kJ, 11–34 g P/d	N	Usual care
Leenders, 2011	U	Type 2 diabetic 30/30	IG: 71, BMI 27·4 CG: 71, BMI 27·2	6 months	3/d, at BF, L, D (IG and CG)	2·5 g Leu. 7·5 g Leu/d	N	3/d, wheat flour placebo
McMurdo, 2009	U	Hospital undernourished 127/126	IG: 81·4, BMI 21·2 CG: 82·2, BMI 21·2	16 weeks	2/d (IG and CG)	200 ml milk-based ONS*. 2520 kJ, 40 g P/d	Y	200 ml skimmed milk ONS*. 840 kJ, 12·4 g P/d
Miller, 2006	U	Hospital, fracture 25/26	IG: 83·5, BMI 21·9 CG: 83·1, BMI 22·1	6 weeks	4/d, during Medpass	145–200 ml milk-based ONS*. 3654–5040 kJ, 34·8–48 g P/d	Y	Attention control and home visits
Myint, 2013	U	Hospital, fracture 61/61	IG: 80·9, BMI 20·2 CG: 81·7, BMI 21·2	28 d	2/d	Approximately 240 ml milk-, whey- or milk and soya-based ONS*. 2090 kJ, 14–19 g P/d. 20–25 µg vit. D, 1·2 g Ca/d	Y	20–25 µg vit. D, 1·2 g Ca/d
Ng, 2015	L	Community Pre-frail and frail 49/50	IG: 69·7, BMI 24·3 CG: 70·1, BMI 23·6	24 weeks	1/d	200 ml milk-based ONS. 1260 kJ, 12 g P/d. 1 mg folate, 29 mg Fe, 200 µg vit. B_{12}, 200 mg vit. B_6, 5 µg vit. D, 0·6 g Ca/d	N	Artificially sweetened equal volume placebo
Payette, 2002	U	Community, frail undernourished 45/46	IG: 81·6, BMI 20·1 CG: 78·6, BMI 20·1	16 weeks	2/d	235 ml milk and soya-based ONS*. 2090–2096 kJ, 18·8–26·6 g P/d	N	Monthly home visits
Persson, 2007	U	Hospital Malnutrition risk 51/57	IG: 85, BMI 19·8 CG: 85, BMI 17·4	4 months	1–2/d	200 ml ONS*. 711–2006 kJ, 8–20 g P. 200 µg folate, 3 µg vit. B_{12}, 5 µg vit. D, 12 mg Zn/d	N	Written dietetic advice
Rondanelli, 2011	U	NH 20/21	IG: 83·5, BMI 21·8 CG: 79·9, BMI 22·1	8 weeks	2/d, at morning and afternoon (IG and CG)	4 g EAA. 300 kJ, 8 g EAA, 2·5 g Leu/d	N	4 g isoenergetic placebo
Rosendahl, 2006	L	Residential care, frail 50/50	IG: 82·9, BMI 24·9 CG: 85·6, BMI 24·5	3 months +3 months F/U	5/2w, after OT (IG and CG)	200 ml milk-based ONS*. 816 kJ, 14·8 g P/d	N	Placebo. 382 kJ, 0·4 g P/d
Schurch, 1998	U	Hospital, fracture 41/41	IG: 81·1, BMI 24·2 CG: 80·2, BMI 24·4	6 months	5/w (IG and CG)	200 ml ONS*. 1050 kJ, 20 g P/d. 1 × 5 mg vit. D	Y	Isoenergetic placebo. 1 × 5 mg vit. D
Smoliner, 2008	U	NH, malnourished or at risk 22/30	IG: 82·2, BMI 21·6 CG: 84, BMI 22·5	12 weeks	1 ONS/d	Milk-based ONS* and enriched foods. 1254 kJ, 20 g P/d from ONS and 5 g/100 ml from soups/sauces	N	Usual care
Sugawara, 2012	U	Stable COPD 18/18	IG: 77·4 CG: 77·1	3 months	2/d	200 ml whey-containing ONS*. 1672 kJ, 20 g P/d	Y	Usual care and diet instructions
Tieland, 2012	L	Community, frail 34/31	IG: 78, BMI 27 CG: 81, BMI 26·2	24 weeks	2/d, at BF, L	250 ml milk-based ONS*. 777 kJ, 30 g P/d	N	2/d, 250 ml 0 g protein placebo
Volkert, 1996	U	Hospital undernourished a: Compliers 11/37, b: Non-compliers 9/37	a: IG compliers: 84·5, BMI 19·8 b: IG non-compliers: 88·7, BMI 19·1 CG: 84·0, BMI 19·3	Approximately 3 weeks hospital + 6 months F/U	2/d, at morning, afternoon. After D/C, 1/d	200 ml ONS*. 2022–2102 kJ, 30 g P/d. After D/C, 200 ml ONS*. 1000–1080 kJ, 16 g P/d	Y	Usual care
Yamada, 2015	U	Community 79/71	IG: 76·3, BMI 22·3 CG: 75·8, BMI 22·7	6 months	1/d	125 ml milk-based ONS*. 836 kJ, 10 g P/d	Y	No treatment
Zak, 2009	L	NH/community Frail 23/23	IG: 78·3, BMI 25·2 CG: 81·1, BMI 25·2	7 weeks	5/w, before exercise (IG and CG)	200 ml milk-based ONS*. 1254 kJ, 12 g P/d	Y	200 ml placebo. 171 kJ, 0·1 g P/d

ROB, risk of bias; U, unclear; L, low or lunch; IG, intervention group; CG, control group; NH, nursing home; MCT, medium-chain TAG; LCT, long-chain TAG; CHF, chronic heart failure; TSP, textured soya protein; COPD, chronic obstructive pulmonary disease; SES, socioeconomic status; TKA, total knee arthroplasty; AD, Alzheimer's disease; F/U, follow up; D, dinner; BF, breakfast; P, protein; OT, occupational therapy; D/C, discharge; EAA, essential amino acids; vit., vitamin; Leu, leucine; ONS, oral nutritional supplement; BCAA, branched chain amino acids; BW, body weight; HP, high protein.

* Intervention (ONS, protein-rich food, etc.) provides additional vitamins and minerals, in addition to protein and kJ.
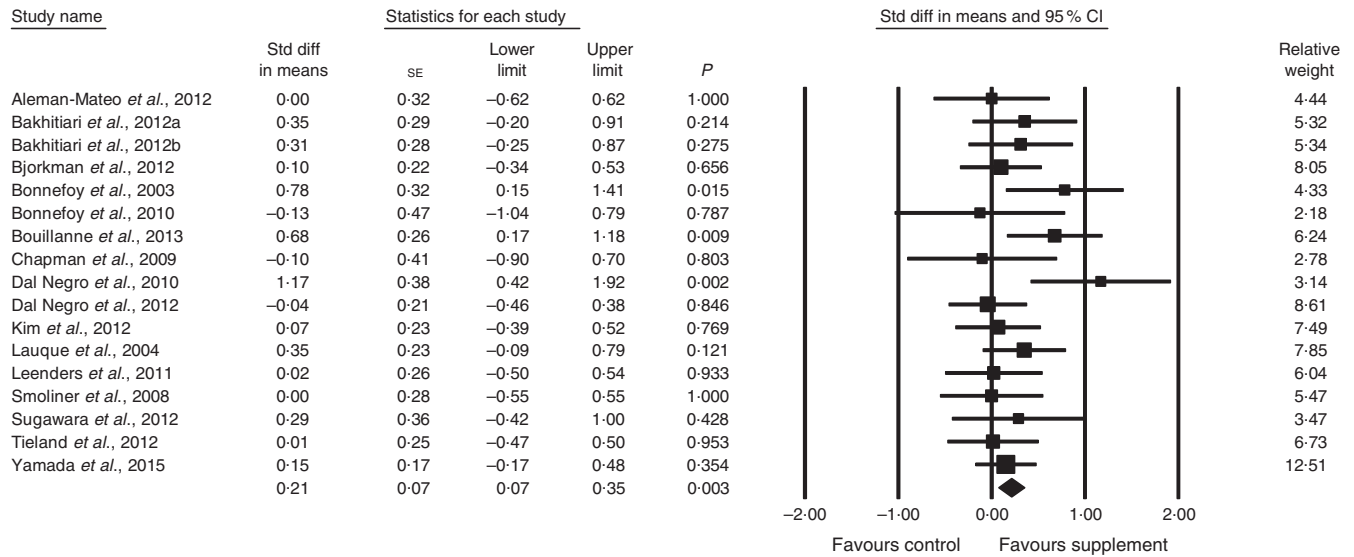
Study name	Std diff in means	SE	Lower limit	Upper limit	P	Std diff in means and 95% CI	Relative weight
Aleman-Mateo et al., 2012	0·00	0·32	−0·62	0·62	1·000		4·44
Bakhitiari et al., 2012a	0·35	0·29	−0·20	0·91	0·214		5·32
Bakhitiari et al., 2012b	0·31	0·28	−0·25	0·87	0·275		5·34
Bjorkman et al., 2012	0·10	0·22	−0·34	0·53	0·656		8·05
Bonnefoy, 2003	0·78	0·32	0·15	1·41	0·015		4·33
Bonnefoy et al., 2010	−0·13	0·47	−1·04	0·79	0·787		2·18
Bouillanne et al., 2013	0·68	0·26	0·17	1·18	0·009		6·24
Chapman et al., 2009	−0·10	0·41	−0·90	0·70	0·803		2·78
Dal Negro et al., 2010	1·17	0·38	0·42	1·92	0·002		3·14
Dal Negro et al., 2012	−0·04	0·21	−0·46	0·38	0·846		8·61
Kim et al., 2012	0·07	0·23	−0·39	0·52	0·769		7·49
Lauque et al., 2004	0·35	0·23	−0·09	0·79	0·121		7·85
Leenders et al., 2011	0·02	0·26	−0·50	0·54	0·933		6·04
Smoliner et al., 2008	0·00	0·28	−0·55	0·55	1·000		5·47
Sugawara et al., 2012	0·29	0·36	−0·42	1·00	0·428		3·47
Tieland et al., 2012	0·01	0·25	−0·47	0·50	0·953		6·73
Yamada et al., 2015	0·15	0·17	−0·17	0·48	0·354		12·51
	0·21	0·07	0·07	0·35	0·003		

Fig. 2. Forest plot of effect of supplements on fat-free mass. Random effects model. Heterogeneity: I^2 15·1, $P = 0·28$.

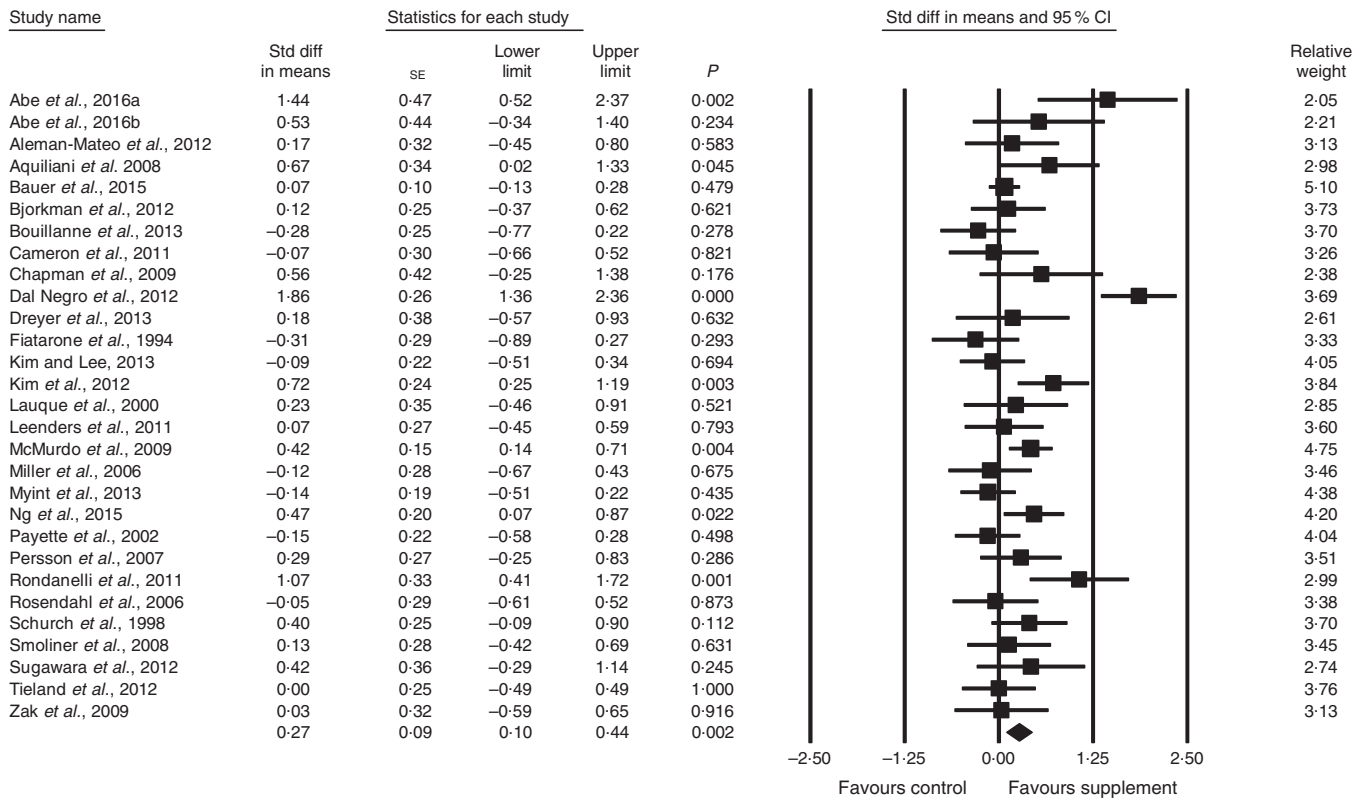| Study name	Std diff in means	SE	Lower limit	Upper limit	P	Std diff in means and 95% CI	Relative weight
Abe et al., 2016a	1·44	0·47	0·52	2·37	0·002		2·05
Abe et al., 2016b	0·53	0·44	−0·34	1·40	0·234		2·21
Aleman-Mateo et al., 2012	0·17	0·32	−0·45	0·80	0·583		3·13
Aquiliani et al. 2008	0·67	0·34	0·02	1·33	0·045		2·98
Bauer et al., 2015	0·07	0·10	−0·13	0·28	0·479		5·10
Bjorkman et al., 2012	0·12	0·25	−0·37	0·62	0·621		3·73
Bouillanne et al., 2013	−0·28	0·25	−0·77	0·22	0·278		3·70
Cameron et al., 2011	−0·07	0·30	−0·66	0·52	0·821		3·26
Chapman et al., 2009	0·56	0·42	−0·25	1·38	0·176		2·38
Dal Negro et al., 2012	1·86	0·26	1·36	2·36	0·000		3·69
Dreyer et al., 2013	0·18	0·38	−0·57	0·93	0·632		2·61
Fiatarone et al., 1994	−0·31	0·29	−0·89	0·27	0·293		3·33
Kim and Lee, 2013	−0·09	0·22	−0·51	0·34	0·694		4·05
Kim et al., 2012	0·72	0·24	0·25	1·19	0·003		3·84
Lauque et al., 2000	0·23	0·35	−0·46	0·91	0·521		2·85
Leenders et al., 2011	0·07	0·27	−0·45	0·59	0·793		3·60
McMurdo et al., 2009	0·42	0·15	0·14	0·71	0·004		4·75
Miller et al., 2006	−0·12	0·28	−0·67	0·43	0·675		3·46
Myint et al., 2013	−0·14	0·19	−0·51	0·22	0·435		4·38
Ng et al., 2015	0·47	0·20	0·07	0·87	0·022		4·20
Payette et al., 2002	−0·15	0·22	−0·58	0·28	0·498		4·04
Persson et al., 2007	0·29	0·27	−0·25	0·83	0·286		3·51
Rondanelli et al., 2011	1·07	0·33	0·41	1·72	0·001		2·99
Rosendahl et al., 2006	−0·05	0·29	−0·61	0·52	0·873		3·38
Schurch et al., 1998	0·40	0·25	−0·09	0·90	0·112		3·70
Smoliner et al., 2008	0·13	0·28	−0·42	0·69	0·631		3·45
Sugawara et al., 2012	0·42	0·36	−0·29	1·14	0·245		2·74
Tieland et al., 2012	0·00	0·25	−0·49	0·49	1·000		3·76
Zak et al., 2009	0·03	0·32	−0·59	0·65	0·916		3·13
	0·27	0·09	0·10	0·44	0·002		

Fig. 3. Forest plot of effect of supplements on muscle strength. Random effects model. Heterogeneity: I^2 68·4, $P < 0·01$.

Muscle strength

A total of twenty-eight studies reported measurements of muscle strength (analysed n 1940), with handgrip strength the most common assessment, followed by leg quadriceps strength. The remaining test measures included were hip and knee, bicep, knee and cycling strength. One study[58] was excluded from analysis, as its SMD results were more than 2 SD from the mean. The effect of supplementation on muscle strength in the twenty-eight studies (Fig. 3) was found to be statistically significant with a small effect size (SMD 0·27; 95% CI 0·10, 0·44, $P < 0·01$, significant heterogeneity, $I^2 = 68·4$, $P < 0·01$). Five studies had low ROB[43,46,56,70,74] and no significant effect on muscle strength was found; however, a small effect was seen in studies with unclear ROB (SMD 0·31; 95% CI 0·10, 0·52, $P < 0·01$, significant heterogeneity, $I^2 = 72·0$, $P < 0·01$).
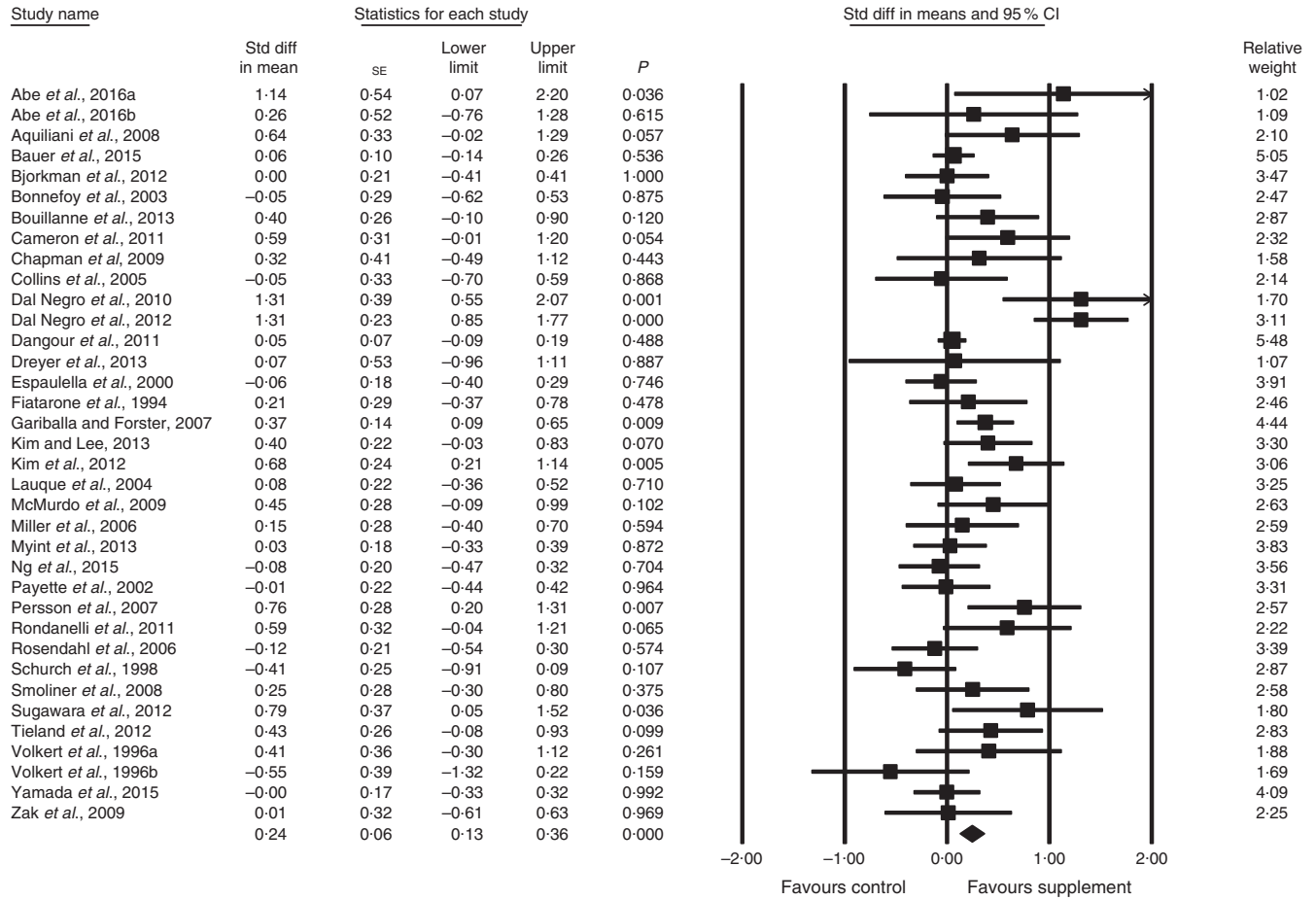
Study name	Std diff in mean	SE	Lower limit	Upper limit	P		Relative weight
Abe *et al.*, 2016a	1·14	0·54	0·07	2·20	0·036		1·02
Abe *et al.*, 2016b	0·26	0·52	−0·76	1·28	0·615		1·09
Aquiliani *et al.*, 2008	0·64	0·33	−0·02	1·29	0·057		2·10
Bauer *et al.*, 2015	0·06	0·10	−0·14	0·26	0·536		5·05
Bjorkman *et al.*, 2012	0·00	0·21	−0·41	0·41	1·000		3·47
Bonnefoy *et al.*, 2003	−0·05	0·29	−0·62	0·53	0·875		2·47
Bouillanne *et al.*, 2013	0·40	0·26	−0·10	0·90	0·120		2·87
Cameron *et al.*, 2011	0·59	0·31	−0·01	1·20	0·054		2·32
Chapman *et al*, 2009	0·32	0·41	−0·49	1·12	0·443		1·58
Collins *et al.*, 2005	−0·05	0·33	−0·70	0·59	0·868		2·14
Dal Negro *et al.*, 2010	1·31	0·39	0·55	2·07	0·001		1·70
Dal Negro *et al.*, 2012	1·31	0·23	0·85	1·77	0·000		3·11
Dangour *et al.*, 2011	0·05	0·07	−0·09	0·19	0·488		5·48
Dreyer *et al.*, 2013	0·07	0·53	−0·96	1·11	0·887		1·07
Espaulella *et al.*, 2000	−0·06	0·18	−0·40	0·29	0·746		3·91
Fiatarone *et al.*, 1994	0·21	0·29	−0·37	0·78	0·478		2·46
Gariballa and Forster, 2007	0·37	0·14	0·09	0·65	0·009		4·44
Kim and Lee, 2013	0·40	0·22	−0·03	0·83	0·070		3·30
Kim *et al.*, 2012	0·68	0·24	0·21	1·14	0·005		3·06
Lauque *et al.*, 2004	0·08	0·22	−0·36	0·52	0·710		3·25
McMurdo *et al.*, 2009	0·45	0·28	−0·09	0·99	0·102		2·63
Miller *et al.*, 2006	0·15	0·28	−0·40	0·70	0·594		2·59
Myint *et al.*, 2013	0·03	0·18	−0·33	0·39	0·872		3·83
Ng *et al.*, 2015	−0·08	0·20	−0·47	0·32	0·704		3·56
Payette *et al.*, 2002	−0·01	0·22	−0·44	0·42	0·964		3·31
Persson *et al.*, 2007	0·76	0·28	0·20	1·31	0·007		2·57
Rondanelli *et al.*, 2011	0·59	0·32	−0·04	1·21	0·065		2·22
Rosendahl *et al.*, 2006	−0·12	0·21	−0·54	0·30	0·574		3·39
Schurch *et al.*, 1998	−0·41	0·25	−0·91	0·09	0·107		2·87
Smoliner *et al.*, 2008	0·25	0·28	−0·30	0·80	0·375		2·58
Sugawara *et al.*, 2012	0·79	0·37	0·05	1·52	0·036		1·80
Tieland *et al.*, 2012	0·43	0·26	−0·08	0·93	0·099		2·83
Volkert *et al.*, 1996a	0·41	0·36	−0·30	1·12	0·261		1·88
Volkert *et al.*, 1996b	−0·55	0·39	−1·32	0·22	0·159		1·69
Yamada *et al.*, 2015	−0·00	0·17	−0·33	0·32	0·992		4·09
Zak *et al.*, 2009	0·01	0·32	−0·61	0·63	0·969		2·25
	0·24	0·06	0·13	0·36	0·000		

Fig. 4. Forest plot of effect of supplements on physical function. Random effects model. Heterogeneity: I^2 56·8, $P < 0·01$.

Group by Supp type	Study name	Std diff in means	SE	Lower limit	Upper limit	P		Relative weight
Amino acid	Abe *et al.*, 2016a	1·44	0·47	0·52	2·37	0·002		10·13
Amino acid	Abe *et al.*, 2016b	0·53	0·44	−0·34	1·40	0·234		10·60
Amino acid	Aquiliani *et al.* 2008	0·67	0·34	0·02	1·33	0·045		12·60
Amino acid	Dal Negro *et al.*, 2012	1·86	0·26	1·36	2·36	0·000		14·08
Amino acid	Dreyer *et al.*, 2013	0·18	0·38	−0·57	0·93	0·632		11·72
Amino acid	Kim *et al.*, 2012	0·72	0·24	0·25	1·19	0·003		14·35
Amino acid	Leenders *et al.*, 2011	0·07	0·27	−0·45	0·59	0·793		13·90
Amino acid	Rondanelli *et al.*, 2011	1·07	0·33	0·41	1·72	0·001		12·63
Amino acid		0·82	0·24	0·35	1·28	0·001		

Fig. 5. Forest plot of effect of essential amino acids on muscle strength. Random effects model: Heterogeneity: I^2 76·9, $P < 0·01$.

Other subgroup analysis showed improvements in studies using EAA (SMD 0·82; 95 % CI 0·35, 1·28, $P < 0·01$, significant heterogeneity, $I^2 = 76·9$, $P < 0·01$) (Fig. 5), but not other supplements used. Studies using supplements with or without additional vitamins or minerals were both effective, with greater effect size seen with supplements with no added vitamins and minerals (SMD 0·77; 95 % CI 0·22, 1·33, $P < 0·01$, significant heterogeneity, $I^2 = 82·3$, $P < 0·01$) than those with added vitamins and minerals (SMD 0·12; 95 % CI 0·00, 0·25, $P = 0·04$, no evidence of heterogeneity, $I^2 = 27·7$, $P = 0·11$). Supplements were effective in studies with undernourished participants in community (SMD 0·55; 95 % CI 0·09, 1·02, $P = 0·02$, significant heterogeneity, $I^2 = 84·6$, $P < 0·01$) or LTC settings (SMD 0·42; 95 % CI 0·05, 0·79, $P = 0·03$, significant heterogeneity, $I^2 = 55·7$, $P = 0·04$); in patients not participating in rehabilitation programs (SMD 0·37; 95 % CI 0·13, 0·61, $P < 0·01$, significant heterogeneity, $I^2 = 75·8$, $P < 0·01$); and in studies measuring upper body muscle strength (online Supplementary Table S3). Meta-regression of the protein (slope −0·001, SE 0·005, $P = 0·78$) or EAA (slope 0·006, SE 0·071, $P = 0·94$) dose provided in
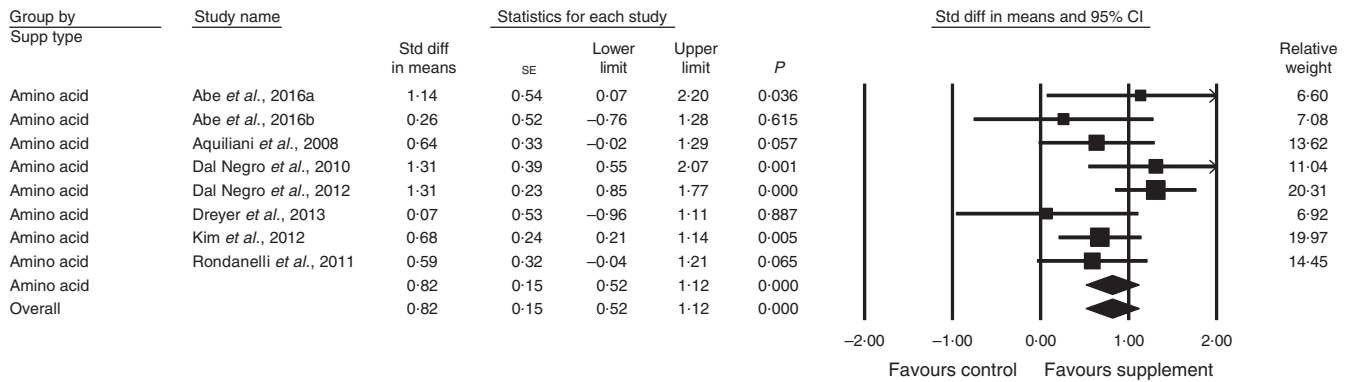
Group by Supp type	Study name	Statistics for each study					Std diff in means and 95% CI	Relative weight
		Std diff in means	SE	Lower limit	Upper limit	P		
Amino acid	Abe et al., 2016a	1·14	0·54	0·07	2·20	0·036		6·60
Amino acid	Abe et al., 2016b	0·26	0·52	−0·76	1·28	0·615		7·08
Amino acid	Aquiliani et al., 2008	0·64	0·33	−0·02	1·29	0·057		13·62
Amino acid	Dal Negro et al., 2010	1·31	0·39	0·55	2·07	0·001		11·04
Amino acid	Dal Negro et al., 2012	1·31	0·23	0·85	1·77	0·000		20·31
Amino acid	Dreyer et al., 2013	0·07	0·53	−0·96	1·11	0·887		6·92
Amino acid	Kim et al., 2012	0·68	0·24	0·21	1·14	0·005		19·97
Amino acid	Rondanelli et al., 2011	0·59	0·32	−0·04	1·21	0·065		14·45
Amino acid		0·82	0·15	0·52	1·12	0·000		
Overall		0·82	0·15	0·52	1·12	0·000		

−2·00   −1·00   0·00   1·00   2·00

Favours control   Favours supplement

Fig. 6. Forest plot of effect of essential amino acids on physical function. Random effects model: Heterogeneity: I^2 33·6, $P = 0.16$.

interventions showed no evidence of dose–response. Duration of intervention also showed no significant effect (slope 0·010, SE 0·006, $P = 0.11$). Studies (n 5) using a true placebo showed a marginally significant effect on muscle strength (SMD 0·67; 95 % CI −0·08, 1·41, $P = 0.08$, significant heterogeneity, $I^2 = 88.4$, $P < 0.01$).

Physical function

In all, thirty-four studies assessed physical function (analysed n 3396) using a variety of methods. Composite physical performance scores included the physical component sections of the Short Form-12 and −36 Health Surveys, the short physical performance battery, Barthel Index or other measures of ADL. Other tests included walking tests, measured for time, distance or speed; and sitting and standing tests. The effect of supplementation on physical function (Fig. 4) was statistically significant with a small effect size (SMD 0·24; 95 % CI 0·13, 0·36, $P < 0.01$, significant heterogeneity, $I^2 = 56.8$, $P < 0.01$). Analysis of seven studies with low ROB[43,46,56,63,64,70,74] indicated no significant effect; however, a small effect was seen in studies with unclear ROB (SMD 0·30; 95 % CI 0·15, 0·45, $P < 0.01$, significant heterogeneity, $I^2 = 60.7$, $P < 0.01$). Other subgroup analysis showed improvements in studies using EAA (SMD 0·82; 95 % CI 0·52, 1·12, $P < 0.01$, no evidence of heterogeneity, $I^2 = 33.6$, $P = 0.16$) (Fig. 6) and moderate protein density ONS (SMD 0·15; 95 % CI 0·00, 0·30, $P < 0.05$, no evidence of heterogeneity, $I^2 = 14.6$, $P = 0.31$), with marginally significant improvement in higher v. lower protein density ONS (SMD 0·35; 95 % CI −0·01, 0·72, $P = 0.06$, no evidence of heterogeneity, $I^2 = 11.9$, $P = 0.32$), but not other supplement types. Studies using supplements with or without additional vitamins or minerals were both effective, with greater effect seen with supplements with no additional vitamins and minerals (SMD 0·83; 95 % CI 0·49, 1·17, $P < 0.01$, no evidence of heterogeneity, $I^2 = 44.3$, $P = 0.11$) compared with supplements with additional vitamins and minerals (SMD 0·13; 95 % CI 0·04, 0·22, $P < 0.01$, no evidence of heterogeneity, $I^2 = 24.0$, $P = 0.12$). Supplements were effective in studies undertaken with undernourished participants in community settings (SMD 0·50; 95 % CI 0·17, 0·82, $P < 0.01$, significant heterogeneity, $I^2 = 76.5$, $P < 0.01$); in patients not participating in rehabilitation programs (SMD 0·31; 95 % CI 0·15, 0·46, $P < 0.01$, significant heterogeneity, $I^2 = 64.9$,

$P < 0.01$); and in studies measuring physical function through usual physical activity tests, the short physical performance battery, and walking tests (online Supplementary Table S3). Meta-regression of the protein (slope 0·006; SE 0·003, $P > 0.05$) or EAA (slope −0·039, SE 0·038, $P = 0.30$) dose provided in interventions showed no evidence of dose–response. Longer intervention length showed marginal effect, with longer study length corresponding to smaller SMD (slope −0·002; SE 0·001, $P = 0.02$). Studies (n 5) using a true placebo showed a significant effect on physical function (SMD 0·67; 95 % CI 0·05, 1·30, $P = 0.04$, significant heterogeneity, $I^2 = 81.6$, $P < 0.01$).

Discussion

Overview

This systematic review and meta-analysis found consistent positive effects, however, significant heterogeneity was evident for the outcomes of muscle strength and physical function, and analysis of only low ROB studies showed no effect on any outcome. The study suggests that supplementation with EAA, and to a lesser extent protein through ONS or protein-rich foods, improves parameters of FFM, muscle strength and physical function for malnourished, frail, sarcopenic, dependent elderly, or those with acute or chronic conditions. This effect was seen even without the addition of RT exercise.

Risk of bias and heterogeneity

Studies with high ROB were excluded from the analysis[82,83], however, only seven of the thirty-nine studies had low ROB. Inadequate or poor reporting of allocation concealment, outcome data and blinding were common issues found. In many studies, the lack of a placebo with the control group receiving no intervention, or usual care, increases the likelihood of bias towards the intervention group.

For trials with low ROB, there was no significant effect for any outcome measure of FFM (one study, n 65), muscle strength (five studies, n 537) or physical function (seven studies, n 706). Subgroup analysis of trials with unclear ROB showed supplements were effective, with a small effect size similar to the overall result (SMD 0·23–0·31 v. SMD 0·21–0·27 for all analysed). This difference may be due to the smaller number

of low ROB studies reducing statistical power, compared with the unclear ROB subgroup (fifteen to twenty-seven studies; *n* 905–2690). In addition, no low ROB studies used EAA, which gave the largest effect in this analysis. The following discussion is based on studies of unclear and low ROB combined.

Most effective supplement

In predetermined subgroup analysis, we found protein-rich foods improved FFM (SMD 0·26), moderate protein density ONS benefitted physical function (SMD 0·15), but EAA produced the largest change in muscle strength and physical function (SMD 0·82, both).

EAA are required for stimulation of MPS[12], with approximately 2·0–2·5 g leucine proposed to be required[7,84]. Five studies using EAA-only formulations, found significant improvements after 6–8 g EAA and 2·5 g leucine/d in predominantly undernourished patients[14–16,45,78]. Leenders *et al.*[80] supplemented leucine only to normally nourished elderly with type 2 diabetes and 1·0 g protein/kg body weight per d, but found no effect of an additional 7·5 g leucine daily. This may be due to patients normal nutritional status, adequate baseline diets, and the threshold of MPS being reached[85]. The result is consistent with recent reviews showing the short term impact of leucine on MPS but not longer term muscle mass or strength effects[86,87].

The large effect of EAA, and smaller effect of ONS and protein-rich foods highlight that the effects of protein consumed as whole food and supplements differ. There are many factors influencing MPS, including the quality and source of the protein ingested. These determine the AA composition, leucine concentration, protein digestibility and subsequent availability of absorbed AA for protein synthesis[12,88–93]. Jonker *et al.* recently found that a high-leucine EAA mixture was superior to a similar mixture with the AA composition of complete proteins, in improving whole-body net protein gain[94], and suggests possible benefits and effectiveness of EAA over complete protein[92].

Protein source influences digestion and absorption rates, and the concept of 'fast' and 'slow' proteins[7,95] has emerged. This differentiates proteins based on digestion and absorption kinetics with rapidly digested and absorbed proteins (e.g. whey protein), producing higher post-prandial serum AA concentrations, compared with more slowly digested and absorbed proteins (e.g. casein)[10,89]. Research shows higher doses of soya and casein are required to stimulate MPS compared with whey, in part due to faster absorption rates of whey protein[95,96].

Food form also contributes to digestibility, with liquid meal replacements[97] providing greater AA and leucine levels than the same formulation ingested as solid food. Minced meat is also superior to whole steak as it is more rapidly digested, absorbed and provides greater improvement in whole-body protein balance in older men[98].

The interventions using protein-rich foods, which found improvement in FFM, utilised a variety of supplements but many used milk or milk powders, which are high quality proteins containing EAA and leucine with a Digestible Indispensable Amino Acid Score of greater than 1·0[99]. In addition, extra protein was used to fortify usual food, likely at meal times

and the dose of protein provided may have reached the threshold for MPS.

The small effect seen with ONS compared with EAA interventions may be due to the quality of the protein and the dose administered by ONS. Most ONS products, although easily absorbed liquids, contain mixtures of whole proteins, for example soya and casein, without additional leucine and may have similar digestibility to 'slow' rather than 'fast' proteins. Further, the co-ingestion of macronutrients such as fat may slow gastrointestinal transit.

Three studies investigated whey protein supplements, containing 20–40 g whey protein with no overall effect seen. Bauer *et al.*[56] reported significant increase in appendicular FFM and improved grip strength in the whey-supplemented group, and Björkman *et al.*[57] found significant reduction in ADL assistance needed in the whey protein group, although not the overall ADL score. Only Sugawara *et al.*[73] found between-group improvements in strength and 6-min walk distance after supplementation with whey containing ONS. Previous studies show approximately 2 g of leucine, in 20 g of whey protein, may be required for MPS[84]; however, only Bauer *et al.*[56] provided adequate information on amounts of leucine provided. Further studies are required to ascertain the effect of whey protein supplements, and authors should provide better information of supplement ingredients.

The largest effect of any supplement was seen with EAA for muscle strength and physical function, but not for FFM. Only three studies using EAA assessed FFM, whereas eight studies assessed muscle strength and physical function, and lack of power may have affected results. However, changes in strength and function, without change in FFM have been found previously. It is known that the correlation between muscle mass and strength reduces with age[100], and studies have shown short-term RT increases strength but not muscle size[101]. The quality of muscle, including muscle composition, morphology, contractile quality and neural innervation also impact strength[101–103], but muscle mass assessments only determine mass and do not account for these other factors[100]. This may contribute to the lack of observed effect of EAA on FFM.

Timing of supplements

With the exception of one study[50], no studies reported quantity of protein consumed per meal or the effect of protein bolus size, and those reporting timing of intake only reported that consumption was during or between meal times. Bouillanne *et al.*[50] investigated 'pulse' (72 % of daily protein at midday meal) compared with 'spread' protein (evenly distributed protein intake, with 30 % of daily protein at midday meal), and found improvements in FFM, but not muscle strength or physical function in undernourished hospitalised patients in the pulse group.

Amount of protein required

Dose–response analysis was conducted and showed no evidence of any effect of daily grams of protein or EAA for any outcome. For EAA, dose varied from 3 to 8 g in the seven

studies assessing the outcome of muscle strength, and 3 to 20 g for the seven studies assessing physical function. The number of studies was small for FFM (*n* 3). Patient disease-related factors and nutrient requirements, as well as baseline functional and nutritional status, are likely to be important in determining any effect; however, we were unable to analyse specific doses further, due to the diversity of interventions, patient health conditions and the lack of adequate information provided by studies.

Subgroup analysis of three studies investigated higher *v.* lower protein density intake with different ONS[59,61,67], and trended towards benefits in physical function from higher energy and protein intake. In these studies, there were differences between the intervention and control group intake, of 8·5–27·6 g protein and 945–1680 kJ (220–400 kcal) energy from supplementation. Marginally beneficial effects on physical function (SMD 0·35, $P = 0.06$), but not muscle strength were still found, suggesting higher protein and energy, with additional micronutrient intake, remains beneficial for physical function. The inclusion of these three studies in the full analysis may have reduced the overall result found for muscle strength and physical function, as the additional protein and nutrients in the lower protein group may have reduced the differences seen between groups.

Contribution of other nutrients

Specific vitamin supplementation was uncommon and most studies provided supplements with small additional amounts of a variety of nutrients, supplied by complete ONS or protein-rich foods containing other nutrients, for example addition of milk protein or ricotta cheese also adds energy, Ca and vitamin B_{12}. Complete ONS contain a complete range of micronutrients, along with energy. Only protein-rich foods, with intrinsic nutrient content, provided a small improvement in FFM; however, no effect was found when only true placebo studies were analysed. For muscle strength and physical function, supplements with and without additional nutrients were both effective. In fact, a greater effect was seen in studies using supplements with no added nutrients, likely due to inclusion of EAA studies in this subgroup, as EAAs independently had the largest effect on muscle strength and physical function. Analysis of studies using a true placebo, found a marginal beneficial effect for muscle strength and a large positive effect for physical function (SMD 0·67, $P = 0.08$ and SMD 0·67, $P = 0.04$), respectively. Four of the five true placebo studies for these analyses used EAA and support the conclusion that EAA rather than other nutrients were the effective component.

There is evidence that vitamin D impacts skeletal muscle. A recent meta-analysis[104] found a small positive effect of vitamin D supplementation on global muscle strength (SMD 0·17) with greater benefit in elders with 25-hydroxyvitamin D level <30 nmol/l. In our meta-analysis, four studies supplemented with vitamin D additional to that intrinsically provided by the supplement, but were not designed to determine effect of vitamin D[56,70,71,79] and we were unable to quantify any effect. Only one study, supplementing with EAA and vitamin D[79] found an improvement in hand grip strength and walk time compared with the no treatment control group.

Energy intake is also important to consider. The provision of additional energy as well as protein was common, particularly if ONS, milk-based drinks or food fortification was used. Protein and EAA are required for cellular functions as well as formation of muscle. However, in times of energy deficit, protein is utilised as an energy source and muscle breakdown occurs[105]. Thus, an energy deficit accelerates muscle protein breakdown and inhibits MPS, which may partly explain why undernourished subjects, most likely in energy and protein deficit, were the most to benefit from supplementation. Additional energy from EAA supplements is unlikely to impact energy intake as the energy provided is only 184–355 kJ (44–85 kcal)/d. In contrast, ONS provided between 623–5040 kJ (149–1200 kcal)/d, but was less effective than EAA in producing an effect on muscle strength and function.

Duration of intervention

Duration of intervention was investigated and a marginal negative effect of longer duration was found for physical function, but no effect was seen for other outcome measures. This suggests intervention time had little or no impact on short-term outcomes, with no blunting of effect due to duration of supplementation. The use of ONS for 6 weeks and 12 months is acceptable to patients and effective in improving both nutritional status and reducing rates of hospital readmissions[19,106]. The time period for improvements in muscle function may be weeks, but continued improvement is unlikely once the individual's threshold of strength and function has been reached. However, additional nutrients may be beneficial to support continued muscle strength and function in the longer term, unless other effective strategies, such as RT are incorporated.

Participants experiencing the greatest benefits

It is important to identify patient groups where interventions are most efficacious. Subgroup analysis of studies in undernourished, but not nourished community-dwelling elderly indicated significant benefits on muscle strength (SMD 0·55) and physical function (SMD 0·50). Muscle strength was also improved in undernourished elderly in LTC (SMD 0·42), indicating that undernourished groups may experience the greatest benefits from supplementation.

Undernourished individuals generally have lower dietary intakes and higher requirements, often due to disease, for example wound/fracture healing, catabolic effects of inflammatory disease or for whole body protein repletion[7,10]. In situations of energy deficit, muscle protein breakdown increases[105] and more protein is needed to compensate for the use of protein as an energy source. Our result is consistent with a Cochrane review finding that undernourished patients benefitted most from supplementation[47]. Nutritional status was defined either by the individual trial authors or by BMI measures, and this is not ideal. Future studies should use current consensus definitions or standard tools to better classify nutritional status, frailty and/or sarcopenia. Few studies reported background dietary intake and this is an important modulator and should be provided in future studies.

Rehabilitation combined with nutrition supplementation

Supplementation with EAA and protein without rehabilitation exercise produced a small effect (SMD 0·23–0·37). We found effect sizes for the outcomes of muscle strength and physical function, but not FFM, were significant in studies without rehabilitation, but not in studies including rehabilitation. Supplements have been shown to augment rehabilitation effects on muscle strength and physical function[73,107], and the lack of effect may be due to the smaller number of studies within the rehabilitation subgroup (*n* 3–12; studies without rehabilitation *n* 13–22).

Concerns related to higher protein intake

The thirty-nine studies included elderly from age 60 to 103 years, and other than mild gastrointestinal upset, no complications were reported. The most common concern related to higher protein intake is kidney function deterioration and this topic has been addressed in recent reviews[7,108]. These suggest 1·0–1·2 g protein/kg body weight per d is suitable for elderly people without a negative impact on kidney function being seen. The International Society of Renal Nutrition and Metabolism[109] recently published guidelines for chronic kidney disease patients, and recommend 1·0 g protein/kg body weight per d for patients with coexisting disease or injury. Given this, additional protein is unlikely to have negative consequences, particularly in undernourished patients. The use of EAA supplements, effective in smaller doses than whole protein-containing foods or ONS, may be a more suitable yet effective alternative to higher protein diets.

Study weights

Individual study weights may have impacted the overall effect size; however, sensitivity analysis that removed individual studies for each outcome showed only a small difference in SMD and marginal change in significance levels, with all SMDs remaining significant. One study, Dal Negro *et al.*[78], with a mid-range weighting of 3·69 for muscle strength, produced the highest individual SMD; when this study was removed during sensitivity analysis, the overall SMD was reduced from 0·27 ($P < 0·01$) to 0·19 ($P < 0·01$). For physical function, removal of both Dal Negro *et al.*[16] (weighting of 1·7) and Dal Negro *et al.*[78] (weighting of 3·11), reduced overall SMD from 0·24 ($P < 0·01$) to 0·17 ($P < 0·01$). Both studies supplemented EAA to sarcopenic patients with chronic obstructive pulmonary disease.

Limitations

As previously discussed, this meta-analysis is limited by the varied study quality with only seven low ROB studies, which limits the strength of the conclusions, and may lead to overestimation of intervention effects. This should be considered in the interpretation of results. Only trials published in English were included and the search strategy used a limit of 65 years and older, which may have eliminated some studies. However, all included papers and reviews found were hand-searched for additional studies. Heterogeneity was significant for analyses of muscle strength

and physical function due to wide variation in outcome measurements, participant characteristics and intervention supplements. Thus, a random effects model was used for all statistical analyses, and pre-determined subgroup analyses according to setting, nutritional status, intervention type and duration of intervention were conducted. Additional post-hoc analysis to determine the effect of true placebo studies, additional nutrients, and dose–response analysis were undertaken.

Conclusions

Given the ROB of the studies, no definitive conclusions can be made regarding the effects of specific supplements. This meta-analysis does however, provide preliminary data suggesting that EAA may be the most effective supplements and undernourished subjects are the most likely to benefit. More studies with low ROB, using a placebo control group, and adequate reporting of intervention characteristics and dietary intakes are needed. Appropriate interventions in this highly vulnerable group of participants will assist in the maintenance or improvement of strength and functional status, and is important from both a clinical and public health perspective.

Supplementary material

For supplementary material/s referred to in this article, please visit https://doi.org/10.1017/S0007114517003816

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