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

Heilok Cheng¹, Jielan Kong¹, Catherine Underwood¹, Peter Petocz², Vasant Hirani¹, Beryl Dawson³ and Fiona O’Leary¹*
¹Nutrition and Dietetics Group, School of Life and Environmental Science, Charles Perkins Centre, University of Sydney, Camperdown, NSW 2006, Australia
²Department of Statistics, Macquarie University, North Ryde, NSW 2109, Australia
³Nutrition and Dietetics, Balmain Hospital, Sydney Local Health District, Balmain, NSW 2041, Australia

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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 trials (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 sarcopenia¹¹, which results in significant disability, mortality and health care costs²,³. 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 conditions⁴. The world’s ageing population is expected to reach 1·5 billion in 2050⁵ 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 sarcopenia⁶,⁷. MPS is stimulated by protein and amino acid (AA) intake⁸,⁹. In older adults, inadequate dietary intake of protein¹⁰ and the decreased anabolic response to dietary protein intake are believed to contribute to the loss of muscle mass⁹,¹¹. Research has further indicated that essential amino acids (EAA) are key regulators of MPS, with leucine a potent muscle stimulator¹²,¹³ and may suppress muscle protein breakdown¹⁰. EAA supplements containing leucine have produced improvements in physical function in some studies¹⁴–¹⁶. Dosage and timing of protein intake may also contribute, with studies¹⁷,¹⁸ demonstrating that protein consumed in ≥30 g bolus intakes are more effective in increasing post-prandial plasma AA concentration, a signal that stimulates MPS¹⁶, and may have the additive effect to overcome the anabolic threshold to MPS¹⁷.

Recent reviews have shown supplements, including high protein oral nutritional supplement (ONS)¹⁹, EAA²⁰ or higher protein diets²¹ improve muscle strength in older adults, but these reviews included healthy and frail elderly participants and included studies using resistance training (RT)²⁰,²². 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

Abbreviations: AA, amino acid; EAA, essential amino acid; FFM, fat-free mass; MPS, muscle protein synthesis; ROB, Risk of Bias; RT, resistance training; SMD, standardised mean difference.

* Corresponding author: F. O’Leary, email fiona.oleary@sydney.edu.au
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 Supplementary 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. The outcome measure chosen for inclusion was based on the characteristics of the instrument, that is tools that measured 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/m\(^2\) 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.

### 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’ effect⁴⁹. 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).
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 (30, 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 patients ($n=488$); 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 dietary 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) (73) to 5040 kJ (1200 kcal) (68), and 4 (73) 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 dietary care. EAA and leucine intake could increase by $3^7$ (79) to 40 g/d (52), and 1–2 $^7$ (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, 60, 75); participants recruited from rehabilitation units (50); participants discharged from hospital to rehabilitation facilities (67, 68, 72); and as novel health interventions (45, 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 (43, 45, 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 (43, 46, 47, 45, 56, 61, 63, 69, 72–74, 78, 80, 81) and outcome assessors in fifteen studies (41, 43, 45, 46, 53, 56, 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, $I^2=0.0$, $P=0.50$) 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

<table>
<thead>
<tr>
<th>First author, year</th>
<th>Study ROB</th>
<th>Population sample size (IG/CG)</th>
<th>Mean age (years), baseline BMI (kg/m²)</th>
<th>Study length</th>
<th>Ingestion details</th>
<th>Dose. Total daily intervention protein and additional nutrients</th>
<th>Rehab (Y/N) Comparator/control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abe, 2016</td>
<td>U</td>
<td>Frail NH residents a: MCT/13/12 b: LCT/13/12</td>
<td>MCT IG: 85.5, BMI 18.7 LCT IG: 87.7, BMI 18.8 CG: 86.8, BMI 19.6</td>
<td>3 months</td>
<td>1/d, at D</td>
<td>a: MCT IG: 6 g MCT, 3 g EAA, 20 µg vit. D, 334 kJ, 1.2 g Leu/d. b: LCT IG: 6 g LCT, 3 g EAA, 20 µg vit. D, 391 kJ, 1.2 g Leu/d</td>
<td>N No treatment</td>
</tr>
<tr>
<td>Aleman-Mateo, 2012</td>
<td>U</td>
<td>Community sarcopenic 20/20</td>
<td>IG: 75.4, BMI 26.5 CG: 76.7, BMI 26.1</td>
<td>3 months</td>
<td>3/d, at BF, L, D</td>
<td>70 g ricotta*, 116.7 kJ, 15.7 g P/d</td>
<td>N Usual diet and home visits</td>
</tr>
<tr>
<td>Aquiliani, 2008</td>
<td>U</td>
<td>Outpatients, CHF 22/22</td>
<td>IG: 74.5, BMI 22.5 CG: 73.1, BMI 23.2</td>
<td>2 months</td>
<td>2/d, at morning, afternoon</td>
<td>4 g EAA. 300 kJ, 8 g EAA, 2.5 g Leu/d</td>
<td>N No treatment</td>
</tr>
<tr>
<td>Bakhtiari, 2012</td>
<td>U</td>
<td>Community a: Soya 25/25 b: TSP 25/25</td>
<td>IG: 73.3, BMI 26.0 TSP IG: 84.6, BMI 27.5 CG: 64.1, BMI 28.5</td>
<td>13 w</td>
<td>3/d (IG and CG)</td>
<td>20 g Leu-enriched whey protein in water, 20 µg vit. D. 1254 kJ, 41 g P, 6 g Leu/d</td>
<td>Y 150 ml juice*, 846 kJ, 0 g P/d</td>
</tr>
<tr>
<td>Bauer, 2015</td>
<td>L</td>
<td>Saropenic 184/186</td>
<td>IG: 78.1, BMI 26.2 CG: 83.0, BMI 24.0</td>
<td>6 months</td>
<td>3/d (IG and CG)</td>
<td>150 ml juice with whey protein*. 1181 kJ, 20 g P/d</td>
<td>N Placebo 1254 kJ, 0 g P/d</td>
</tr>
<tr>
<td>Björkman, 2012</td>
<td>U</td>
<td>Retirement home frail 30/27</td>
<td>IG: 83.7, BMI 27.3 CG: 83.2 BMI 27.3</td>
<td>9 months</td>
<td>2/d, at morning, afternoon</td>
<td>200 ml ONS*. 1686 kJ, 30 g P/d</td>
<td>N 0 ml placebo</td>
</tr>
<tr>
<td>Bonnefoy, 2010</td>
<td>U</td>
<td>Malnourished catabolic 15/15</td>
<td>IG: 82.5, BMI 22.6 CG: 79.4, BMI 23.4</td>
<td>2 weeks</td>
<td>3–5/d, at L, D</td>
<td>6.5–9 g P (47.5% BCAA). 213–357 kJ, 11–16 g P, 2.6–4 g Leu/d. Added to food</td>
<td>N Usual dietetic advice</td>
</tr>
<tr>
<td>Bouillanne, 2010</td>
<td>U</td>
<td>Outpatients sarcopenic COPD 16/16</td>
<td>IG: 78.5, BMI 20.0 CG: 73.0, BMI 20.1 All participants 65–67.9</td>
<td>12 weeks</td>
<td>2/d, at morning and afternoon (IG and CG)</td>
<td>8 g EAA, 183 kJ, 8 g EAA, 2.5 g Leu/d</td>
<td>N Placebo</td>
</tr>
<tr>
<td>Collins, 2005</td>
<td>U</td>
<td>NH, wound clinic 18/20</td>
<td>IG: 79.2, BMI 25.5 CG: 81.0, BMI 26.4 IG: 75.0, BMI 20.2 CG: 75.0, BMI 20.2</td>
<td>4 weeks</td>
<td>3/d, with meals (IG and CG)</td>
<td>80 ml, 8.4 kJ (2 kcal/ml) ONS*. 1995 kJ, 19 g P/d</td>
<td>N 80 ml, 4.2 kJ (1 kcal/ml) ONS*. 1050 kJ, 8.8 g P/d</td>
</tr>
<tr>
<td>Dal Negro, 2012</td>
<td>U</td>
<td>Outpatients sarcopenic COPD 44/44</td>
<td>IG: 75.0, BMI 20.0 CG: 73.0, BMI 20.1 All participants 65–67.9</td>
<td>12 weeks</td>
<td>2/d, at morning and afternoon (IG and CG)</td>
<td>4 g EAA. 183 kJ, 8 g EAA, 2.5 g Leu/d</td>
<td>N Placebo</td>
</tr>
<tr>
<td>Dangour, 2011</td>
<td>U</td>
<td>Community Low-middle SES 502/504</td>
<td>IG: 68.0, BMI 34.0 CG: 70.0, BMI 29.0</td>
<td>24 months</td>
<td>1/d</td>
<td>50 g vegetable powder* and 50 g low lactose milk powder*. 1685 kJ, 15.5 g P/d</td>
<td>N No treatment</td>
</tr>
<tr>
<td>Dreyer, 2013</td>
<td>U</td>
<td>Post-surgical, TKA 21/19</td>
<td>IG: 82.4, BMI 25.4 CG: 82.7, BMI 25.4 IG: 85.7, BMI 25.4 CG: 89.2, BMI 25.8</td>
<td>60 d + 6 months</td>
<td>2/d, at morning and afternoon (IG and CG)</td>
<td>20 g EAA. 7.2 g Leu/d</td>
<td>Y 2/d, 20 g alanine</td>
</tr>
<tr>
<td>Espaulella, 2000</td>
<td>L</td>
<td>Inpatients, fracture 85/86</td>
<td>IG: 82.4, BMI 25.4 CG: 82.7, BMI 25.4 IG: 85.7, BMI 25.4 CG: 89.2, BMI 25.8</td>
<td>60 d + 6 months</td>
<td>1/d (IG and CG)</td>
<td>200 ml ONS*. 623 kJ, 20 g P/d</td>
<td>N 200 ml placebo. 648 kJ, 0 g P/d</td>
</tr>
<tr>
<td>Fiatarone, 1994</td>
<td>U</td>
<td>NH, frail 24/26 Hospital 106/119</td>
<td>IG: 75.5, BMI 25.2 CG: 75.3, BMI 25.2 IG: 79.2, BMI 18.9 CG: 78.7, BMI 18.8 IG: 84.5, BMI 22.3 CG: 84.7, BMI 21.8</td>
<td>6 weeks + 6 months</td>
<td>2/d, at morning and afternoon (IG and CG)</td>
<td>200 ml ONS*. 4160 kJ, 49.8 g P/d</td>
<td>N 251 ml placebo</td>
</tr>
<tr>
<td>Gariballa, 2007</td>
<td>L</td>
<td>Community sarcopenic 39/39</td>
<td>IG: 79.2, BMI 19.9 CG: 78.7, BMI 18.8 IG: 84.6, BMI 22.3 CG: 84.7, BMI 21.8</td>
<td>12 weeks</td>
<td>2/d</td>
<td>3 g EAA. 6 g EAA, 2.5 g Leu/d</td>
<td>N Health education classes</td>
</tr>
<tr>
<td>Kim, 2012</td>
<td>U</td>
<td>Community frail 43/44</td>
<td>IG: 84.6, BMI 22.3 CG: 84.7, BMI 21.8</td>
<td>60 d</td>
<td>1–2/d, with meals</td>
<td>150–200 ml milk-, whey- or milk and pea protein-based ONS*. 1254–2090 kJ, 24–39 g P/d</td>
<td>N No treatment</td>
</tr>
</tbody>
</table>
Table 1. Continued

<table>
<thead>
<tr>
<th>First author, year</th>
<th>Study ROB</th>
<th>Population sample size (IG/C.G)</th>
<th>Mean age (years), baseline BMI (kg/m²)</th>
<th>Study length</th>
<th>Ingestion details</th>
<th>Dose. Total daily intervention protein and additional nutrients</th>
<th>Rehab (Y/N)</th>
<th>Comparator/control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lauque, 2004 U</td>
<td>Hospital, AD care 46/45</td>
<td>IG: 79, BMI 22.2 CG: 78, BMI 22.6</td>
<td>3 months</td>
<td>1–2/d</td>
<td>150–200 ml milk- or milk and pea protein-based ONS*. 1254–2090 kJ, 11–34 g P/d</td>
<td>N</td>
<td>Usual care</td>
<td></td>
</tr>
<tr>
<td>Leenders, 2011 U</td>
<td>Type 2 diabetic 30/30</td>
<td>IG: 71, BMI 27.4 CG: 71, BMI 27.2</td>
<td>6 months</td>
<td>3/d, at BF, L, D (IG and CG)</td>
<td>2–5 g Leu. 7 g Leu/d</td>
<td>N</td>
<td>3/d, wheat flour placebo</td>
<td></td>
</tr>
<tr>
<td>McMurdo, 2009 U</td>
<td>Hospital undernourished 127/126</td>
<td>IG: 84.1, BMI 21.2 CG: 82.2, BMI 21.2</td>
<td>16 weeks</td>
<td>2/d (IG and CG)</td>
<td>200 ml milk-based ONS*. 2520 kJ, 40 g P/d</td>
<td>Y</td>
<td>200 ml skimmed milk ONS*. 840 kJ, 12.4 g P/d</td>
<td></td>
</tr>
<tr>
<td>Miller, 2006 U</td>
<td>Hospital, fracture 25/26</td>
<td>IG: 83.5, BMI 21.9 CG: 83.1, BMI 22.1</td>
<td>6 weeks</td>
<td>4/d, during Medpass</td>
<td>145–200 ml milk-based ONS*. 3654–5040 kJ, 34.8–48 g P/d</td>
<td>Y</td>
<td>Attention control and home visits</td>
<td></td>
</tr>
<tr>
<td>Myint, 2013 U</td>
<td>Hospital, fracture 61/61</td>
<td>IG: 80.9, BMI 20.2 CG: 81.7, BMI 21.2</td>
<td>28 d</td>
<td>2/d</td>
<td>Approximately 240 ml milk-, whey- or soya-based ONS*. 2090 kJ, 14–19 g P/d, 20–25 μg vit. D, 1–2 g Ca/d</td>
<td>Y</td>
<td>20–25 μg vit. D, 1.2 g Ca/d</td>
<td></td>
</tr>
<tr>
<td>Ng, 2015 L</td>
<td>Community 49/50</td>
<td>IG: 69.7, BMI 24.3 CG: 70.1, BMI 23.6</td>
<td>24 weeks</td>
<td>1/d</td>
<td>200 ml milk-based ONS. 1250 kJ, 12 g P/d, 1 mg folate, 29 mg Fe, 200 μg vit. B₁₂, 200 mg vit. B₆, 5 μg vit. D, 0.6 g Ca/d</td>
<td>N</td>
<td>Artificially sweetened equal volume placebo</td>
<td></td>
</tr>
<tr>
<td>Payette, 2002 U</td>
<td>Community, frail 45/46</td>
<td>IG: 81.6, BMI 20.1 CG: 78.6, BMI 20.1</td>
<td>16 weeks</td>
<td>2/d</td>
<td>235 ml milk and soya-based ONS*. 2090–2096 kJ, 18.8–26 g P/d</td>
<td>N</td>
<td>Monthly home visits</td>
<td></td>
</tr>
<tr>
<td>Persson, 2007 U</td>
<td>Hospital 51/57</td>
<td>IG: 85, BMI 19.8 CG: 85, BMI 17.4</td>
<td>4 months</td>
<td>1–2/d</td>
<td>200 ml ONS*. 711–2006 kJ, 8–20 g P, 200 μg folate, 3 μg vit. B₁₂, 5 μg vit. D, 12 mg Zn/d</td>
<td>N</td>
<td>Written dietetic advice</td>
<td></td>
</tr>
<tr>
<td>Rondanelli, 2011 U</td>
<td>NH 20/21</td>
<td>IG: 83.5, BMI 21.8 CG: 79.9, BMI 22.1</td>
<td>8 weeks</td>
<td>2/d, at morning and afternoon (IG and CG)</td>
<td>4 g EAA. 300 kJ, 8 g EAA, 2.5 g Leu/d</td>
<td>N</td>
<td>4 g isoenergetic placebo</td>
<td></td>
</tr>
<tr>
<td>Rosendahl, 2006 L</td>
<td>Residential care, frail 50/50</td>
<td>IG: 82.9, BMI 24.9 CG: 85.6, BMI 24.5</td>
<td>3 months</td>
<td>5/2w, after OT (IG and CG)</td>
<td>200 ml milk-based ONS*. 816 kJ, 14.8 g P/d</td>
<td>N</td>
<td>Placebo. 382 kJ, 0.4 g P/d</td>
<td></td>
</tr>
<tr>
<td>Schuch, 1996 U</td>
<td>Hospital, fracture 41/41</td>
<td>IG: 81.1, BMI 24.2 CG: 80.2, BMI 24.4</td>
<td>6 months</td>
<td>5w (IG and CG)</td>
<td>200 ml ONS*. 1050 kJ, 20 g P, 1 × 5 mg vit. D</td>
<td>Y</td>
<td>Isoenergetic placebo. 1 × 5 mg vit. D</td>
<td></td>
</tr>
<tr>
<td>Smoliner, 2008 U</td>
<td>NH, malnourished or at risk 23/20</td>
<td>IG: 82.2, BMI 21.6 CG: 84, BMI 22.5</td>
<td>12 weeks</td>
<td>1 ONS/d</td>
<td>Milk-based ONS* and enriched foods. 1254 kJ, 20 g P/d from ONS and 5 g/100 ml from soups/sauces</td>
<td>N</td>
<td>Usual care</td>
<td></td>
</tr>
<tr>
<td>Sugawara, 2012 U</td>
<td>Stable COPD 18/18</td>
<td>IG: 77.4 CG: 77.1</td>
<td>3 months</td>
<td>2/d</td>
<td>200 ml whey-containing ONS*. 1672 kJ, 20 g P/d</td>
<td>Y</td>
<td>Usual care and diet instructions</td>
<td></td>
</tr>
<tr>
<td>Tieland, 2012 L</td>
<td>Community, frail 34/31</td>
<td>IG: 78, BMI 27.1 CG: 81, BMI 26.2</td>
<td>24 weeks</td>
<td>2/d, at BF, L</td>
<td>250 ml milk-based ONS*. 777 kJ, 30 g P/d</td>
<td>N</td>
<td>2/d, 250 ml 0 g protein placebo</td>
<td></td>
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<tr>
<td>Yamada, 2015 U</td>
<td>Community 79/71 NH/community 23/23</td>
<td>IG: 76.3, BMI 22.3 CG: 75.8, BMI 22.7</td>
<td>6 months</td>
<td>1/d</td>
<td>125 ml milk-based ONS*. 836 kJ, 10 g P/d</td>
<td>Y</td>
<td>No treatment</td>
<td></td>
</tr>
<tr>
<td>Zak, 2009 L</td>
<td>Frail 23/23</td>
<td>IG: 78.3, BMI 25.2 CG: 81.1, BMI 25.2</td>
<td>5 weeks, before exercise (IG and CG)</td>
<td>200 ml milk-based ONS*. 1254 kJ, 12 g P/d</td>
<td>200 ml placebo. 171 kJ, 0.1 g P/d</td>
<td>Y</td>
<td></td>
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</table>

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.
Effect of protein and amino acid supplements

<table>
<thead>
<tr>
<th>Study name</th>
<th>Std diff in means</th>
<th>Std diff in means and 95 % CI</th>
<th>Relative weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abe et al., 2016a</td>
<td>1.44</td>
<td>0.47 - 2.37</td>
<td>2.05</td>
</tr>
<tr>
<td>Abe et al., 2016b</td>
<td>0.53</td>
<td>0.44 - 1.40</td>
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<td>Alevan-Mateo et al., 2012</td>
<td>0.17</td>
<td>0.32 - 0.80</td>
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<tr>
<td>Aquilini et al., 2008</td>
<td>0.67</td>
<td>0.52 - 1.33</td>
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<tr>
<td>Bauer et al., 2015</td>
<td>0.07</td>
<td>0.10 - 0.28</td>
<td>5.10</td>
</tr>
<tr>
<td>Bjorkman et al., 2012</td>
<td>0.12</td>
<td>0.25 - 0.62</td>
<td>3.75</td>
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<tr>
<td>Bouillanne et al., 2013</td>
<td>-0.28</td>
<td>-0.77 - 0.22</td>
<td>3.69</td>
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<td>Cameron et al., 2011</td>
<td>-0.30</td>
<td>-0.66 - 0.52</td>
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<td>Chapman et al., 2011</td>
<td>0.56</td>
<td>0.26 - 1.38</td>
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<td>Dal Negro et al., 2012</td>
<td>1.86</td>
<td>0.26 - 2.36</td>
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<td>0.18</td>
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<td>Fiatarone et al., 1994</td>
<td>-0.31</td>
<td>-0.89 - 0.27</td>
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<td>Kim and Lee, 2013</td>
<td>-0.09</td>
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<td>0.33 - 1.72</td>
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<td>Rosendahl et al., 2006</td>
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<td>0.29 - 0.61</td>
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<td>Schurch et al., 1998</td>
<td>0.40</td>
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<td>Smoliner et al., 2008</td>
<td>0.13</td>
<td>0.28 - 0.42</td>
<td>3.45</td>
</tr>
<tr>
<td>Sugawara et al., 2012</td>
<td>0.42</td>
<td>0.36 - 0.93</td>
<td>2.74</td>
</tr>
<tr>
<td>Tieland et al., 2012</td>
<td>0.00</td>
<td>0.20 - 0.49</td>
<td>3.76</td>
</tr>
<tr>
<td>Zak et al., 2009</td>
<td>0.03</td>
<td>0.32 - 0.65</td>
<td>3.13</td>
</tr>
</tbody>
</table>

Fig. 2. Forest plot of effect of supplements on fat-free mass. Random effects model. Heterogeneity: $I^2 = 15.1, P = 0.28$.

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\(^5\) 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 (SMD 0.43, 0.46, 0.50, 0.70, 0.74) 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, $I^2 = 72.0$, $P < 0.01$).

Fig. 3. Forest plot of effect of supplements on muscle strength. Random effects model. Heterogeneity: $I^2 = 68.4$, $P < 0.01$.\(^5\)
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 and minerals (SMD 0.77; 95% CI 0.22, 1.35, 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 S5). 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
interventions showed no evidence of dose–response. Duration of intervention also showed no significant effect (slope 0.010, 95% CI −0.006, 0.011). Studies (n 7) 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, $I^2 = 38$%). Studies with high ROB were excluded from the analysis (82, 83), with marginally significant effect (slope 0.06, 95% CI 0.0, 0.12). Supplements with additional vitamins and minerals were both effective, with greater effect seen with supplements with no additional vitamins and minerals (SMD 0.82; 95% CI 0.59, 1.21, P = 0.04, significant heterogeneity, $I^2 = 81$%).

### Physical function

In all, thirty-four studies assessed physical function (analysed n 3596) 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, $I^2 = 56$%). Analysis of seven studies with low ROB (24, 44, 46, 64, 70, 71) 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, $I^2 = 60$%). 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, $I^2 = 33$%) and moderate protein density ONS (SMD 0.15; 95% CI 0.00, 0.30, P < 0.05, no evidence of heterogeneity, $I^2 = 14$%), 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, $I^2 = 11$%), 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, $I^2 = 44$%). Supplements were effective in studies undertaken with undernourished participants in community settings (SMD 0.50; 95% CI 0.04, 0.22, P < 0.01, no evidence of heterogeneity, $I^2 = 24$, P = 0.12). Supplements were effective in studies undertaken with undernourished participants in community settings (SMD 0.50; 95% CI 0.04, 0.22, P < 0.01, no evidence of heterogeneity, $I^2 = 24$, P = 0.12). Supplements were effective in studies undertaken with undernourished participants in community settings (SMD 0.50; 95% CI 0.04, 0.22, P < 0.01, no evidence of heterogeneity, $I^2 = 24$, P = 0.12). Supplements were effective in studies undertaken with undernourished participants in community settings (SMD 0.50; 95% CI 0.04, 0.22, P < 0.01, no evidence of heterogeneity, $I^2 = 24$, P = 0.12). Supplements were effective in studies undertaken with undernourished participants in community settings (SMD 0.50; 95% CI 0.04, 0.22, P < 0.01, no evidence of heterogeneity, $I^2 = 24$, P = 0.12). Supplements were effective in studies undertaken with undernourished participants in community settings (SMD 0.50; 95% CI 0.04, 0.22, P < 0.01, no evidence of heterogeneity, $I^2 = 24$, P = 0.12).

### 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 0.23–0.51). This difference may be due to the smaller number of trials. The analysis showed no evidence of dose–response and no effect for trials with low ROB.
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.(90) 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(96,87).

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(12,98–93). 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, 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(101,102), but muscle mass assessments only determine mass and do not account for these other factors(102). 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 \( r \) lower protein density intake with different ONS\(^{[56,70,71,79]} \), 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, \( P = 006 \)), 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 EAs 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, \( P = 008 \) 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 \(<30 \text{nmol/l} \). 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\(^{[35,106]} \). 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 replenishment\(^{[7,10]} \). 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 0.25–0.37). 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 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.[106] (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.

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Supplementary material

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

References

Effect of protein and amino acid supplements


Effect of protein and amino acid supplements


