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The effect of protein supplements on functional frailty in older persons: A systematic review and meta-analysis

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ABSTRACT

Background: The effect of protein supplementation in attenuating loss of muscle mass, strength and function in community-dwelling older people has been promising, however, its benefits in pre-frail and frail older people remains unclear.

Objective: To determine the effect of protein supplementation on muscle mass, strength and function in frail older people by reviewing and conducting meta-analysis of relevant randomized controlled trials (RCTs).

Design: This review was registered at PROSPERO (CRD42017079276) and conducted according to Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) guidelines. Using a pre-determined e-search strategy, we searched PubMed, Medline, EMBASE, CINAHL, LILACS, Web of Science, Cochrane and Scopus databases. Inclusion criteria were RCTs that assessed the effect of protein supplementation on muscle mass, strength and function in frail individuals aged ≥65 years. The main outcomes were lean body mass (LBM), handgrip, leg extension, leg press strength, short physical performance battery (SPPB) score, and gait velocity.

Results: Of the eight studies included in this review, 503 subjects were enrolled and four different protein supplements were assessed. Despite the variation in methodology, studies were homogenous with I-squared < 10.0%. The meta-analysis showed no significant effect of protein supplementation on LBM (mean difference 1.17 kg, 95% CI: −1.97–4.3), handgrip (mean difference 0.15, 95% CI: −0.95–1.24), leg extension (mean difference −3.68 kg, 95% CI: −12.72–5.36), leg press (mean standardized difference 0.26 kg, 95% CI: −0.30–0.82), SPPB (mean difference 0.61, 95% CI: −0.02–1.23), or gait velocity (mean difference -0.20 m/s, 95% CI: −0.95–0.55).

Conclusion: Protein supplementation alone does not significantly improve muscle mass, strength or function in pre-frail or frail older people.

1. Introduction

Adults aged 65 years and older comprised 8.5% of the global population in 2015; a proportion that is expected to triple by 2050 (He, Goodkind, & Kowal, 2015). Frailty is commonly accepted as a geriatric syndrome characterized by increased vulnerability to stressors due to age-related decline in physiological systems (Fried et al., 2001; Thompson, Theou, Karnon, Adams, & Visvanathan, 2018; Topinkova, 2008; Xue, 2011). Frailty involves multiple organs and systems, and increases the risk of falls, hospitalization, institutionalization, morbidity and mortality (Buckinx et al., 2015; Litchford, 2014). Fried et al. (2001) defined frailty according to five components: unintentional weight loss, weakness, self-reported exhaustion, slow walking speed, and low physical activity. Another disease process that is closely related to but distinct from frailty is sarcopenia. Sarcopenia is defined as the progressive age-related loss of muscle mass, strength and/or function (Bernabei et al., 2015; Rolland et al., 2008). Sarcopenia comprises the physical components of frailty including muscle mass, strength and function (Bernabei et al., 2015). The decline in muscle mass occurs after the age of 30 years at around 3–5% per decade, a rate that accelerates

Abbreviations: BCAA, branched-chain amino acid; DXA, dual-energy X-ray absorptiometry; EAA, essential amino acid; FFC, fried frailty criteria; FWG, frailty working group; HMB, beta-hydroxy-beta-methylbutyrate; MPS, muscle protein synthesis; RCT, randomized controlled trial; SD, standard deviation; SPPB, short physical performance battery

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after the age of 60 (Holloszy, 2000; Melton, 2000). The etiology of sarcopenia is multifactorial, with lifestyle and nutritional factors having crucial roles (Rolland et al., 2008).

The maintenance of muscle mass relies on a dynamic equilibrium between muscle protein synthesis (MPS) and breakdown (Breen & Phillips, 2011). One review showed that older individuals had blunted MPS responses to low dose amino acids compared with younger persons (Breen & Phillips, 2011). Therefore, the consumption of adequate dietary protein that contains essential amino acids such as meat, poultry, fish and dairy products is imperative (Australian Government Department of Health & Ageing; New Zealand Ministry of Health, 2006; Landi et al., 2016). This is required to prevent negative nitrogen balance, to delay or prevent the development of sarcopenia, and to maintain energy balance, physical function and quality of life (Calvani et al., 2013; Morais, Chevalier, & Gougeon, 2006; Wilson, Purushothaman, & Morley, 2002; Wolfe, 2012). Observational studies and randomized controlled trials (RCTs) showed that consumption of high dietary protein intake resulted in the deceleration of loss of muscle mass and the maintenance of muscle mass and function (Bartali et al., 2006; Dirks et al., 2017; Houston et al., 2008; Tieland, van de Rest et al., 2012). A minimum of 1.0–1.2 g of protein per kg per day for individuals aged over 65 is recommended to ensure adequate nutritional intake (Bauer et al., 2013).

Despite some studies showing protein supplementation may attenuate measures of sarcopenia in frail older people, RCTs showed inconsistent results (Dirks et al., 2017; Fiatarone et al., 1994; Hankey, Summerbell, & Wynne, 1993; Ikeda et al., 2016; Kim & Lee, 2013; Payette, Boutier, Coulombe, & Gray-Donald, 2002; Tieland, Dirks et al., 2012; Tieland, van de Rest et al., 2012). Furthermore, one meta-analysis that assessed the effect of protein supplementation in the general older population hypothesized the potential benefit of protein supplementation in the frail population (Tieland et al., 2017). It is therefore necessary to determine whether protein supplementation has an effect in attenuating frailty syndrome.

We therefore hypothesized that protein supplementation alone attenuates the decline in measures of muscle mass, strength and function in pre-frail and frail older people. We evaluated available RCTs where forms of oral protein supplements were administered alone with or without concomitant exercise intervention, provided that both control and intervention group received the same exercise program. We conducted meta-analyses to quantify the effect of protein supplementation on lean body mass, strength and function, which are the main components of physical frailty.

2. Methods

2.1. Search strategy

This review was registered at PROSPERO (University of York) with registration number CRD42017079276 and conducted according to the Preferred Reporting Items for Systematic Review and Meta-Analyses (PRISMA) guidelines.

Systematic literature search was conducted in PubMed, MEDLINE, EMBASE, EMCARE, CINAHL, LILACS, Web of Science, the Cochrane Central Registry of Controlled Trials (CENTRAL) and Scopus from inception of the database until July 2019. Based on a discussion with a librarian at the University of Melbourne, the following terms were included in the literature search: protein supplement* OR oral supplement* OR nutritional supplement* OR dietary supplement* OR amino acid OR leucine OR HMB OR beta-hydroxy-beta-methylbutyrate, frail* OR sarcopeni*. An example of a search strategy developed for MEDLINE (via Ovid) can be found in Supplemental Table 1. The search was restricted to articles published in English. Bibliographic search was conducted by reviewing the reference lists of the included studies or key texts. Where primary data was not available in full-text, we attempted to contact the authors twice over a two-week period. A study was then

Table 1
Characteristics of the studies included.

Author, Year	Study design	Eligibility criteria	Sample size	Intervention	Control	Protein amount (g/d)	Duration (weeks)
Dirks et al. (2017) (20)	RCT	≥ 65, pre-frail or frail according to FFC	34	2 x 250 ml MPC daily; exercise program	2 x 250 ml placebo drinks daily; exercise program	30	24
Fiatarone et al. (1994) (26)	RCT	> 70, residential status in aged care facilities, able to walk ≥ 6 m	100	1 x 240 ml soy-based drink daily	1 x 240 ml placebo drink daily	Approx. 15.3	10
Hankey et al. (1993) (27)	RCT	> 75, frailty screening tool not specified	20	1 x Build – Up (Clintec and glucose polymer drink	None	Approx. 30	8
Ikeda et al. (2016) (24)	Crossover RCT	≥ 65, pre-frail or frail according to FFC	52	6 g amino acid supplement containing BCAA and EAAs twice weekly after exercise	6 g maltodextrin twice weekly after exercise	Approx. 6 g/ week	24 + 4 weeks washout period
Kim and Lee (2013)(25)	RCT	≥ 65, frail according to FWG focusing on mobility and nutrition	87	2 x 200 ml commercial nutritional drink daily, encouragement to increase food intake	Monthly visit with a small gift	25	12
Payette et al., 2002 (23)	RCT	> 65, involuntary weight loss of > 5%, > 7.5% or > 10% in the past month, 3 months or 6 months respectively and BMI < 24	83	2 x 235 ml either Ensure® or Ensure® Plus daily, monthly visit and encouragement to increase food intake	Monthly visit with a small gift	Approx. 32	16
Tieland, van de Rest et al. (2012) (19)	RCT	≥ 65, pre-frail or frail according to FFC	65	2 x 250 ml MPC daily	2 x 250 ml placebo drinks daily	30	24
Tieland, van de Rest et al. (2012) (22)	RCT	≥ 65, pre-frail or frail according to FFC	62	2 x 250 ml MPC daily; exercise program	2 x 250 ml placebo drinks daily; exercise program	30	24

Abbreviations: Approx. = approximately; BCAA = branched-chain amino acid; EAA = essential amino acids; FFC = Fried frailty criteria; FWG = frailty working group; g/d = gram(s)/day; MPC = milk protein concentrate.

excluded if there was no response from authors and key data was absent from the study.

2.2. Inclusion and exclusion criteria

Search results from each database were exported into EndNote X8.0.1. Duplicate publications were removed, and the remaining results were screened based on the following inclusion criteria:

- RCTs that compared protein supplementation to placebo in pre-frail or frail older persons;
- RCTs that compared different amounts of protein supplementation;
- RCTs that compared different types of protein supplementation;
- RCTs that compared different timings of the administration of protein supplementation with measures of physical performance, muscle mass and strength, weight management, and number of falls and fractures;
- RCTs that included frailty in their keywords and as part of their inclusion criteria, despite having not mentioned any specific frailty screening tools;
- Protein supplementation must be in the form of oral dietary supplements that can be in a form of dairy products, meat, or nutritional supplements e.g. Ensure®, Sustagen®, leucine, beta-hydroxy beta-methylbutyrate (HMB), or whey protein;
- The population must be ≥65-year-old and meet the criteria of frailty syndrome as determined by a frailty screening tool (e.g. Fried Frailty Criteria (FFC) and Frailty Working Group (FWG)) or admission in residential care.

Exclusion criteria included:

- RCTs that combined both exercise program and protein supplementation or that use the combination of protein and other macronutrient or micronutrient as intervention, except where both control and intervention groups received identical additional intervention(s) and protein supplementation being the only variable between both groups e.g. identical exercise program or identical additional interventions for both control and intervention group.
- Participants who were not assessed with frailty screening tools as part of inclusion criteria.
- Healthy older subjects or individuals who were sarcopenic or at risk of malnutrition but not screened using a validated frailty screening tool.
- Participants who were acutely ill or had chronic diseases e.g. dementia, chronic liver disease, renal failure, diabetes mellitus or were included as part of rehabilitation program post-operatively.

2.3. Data extraction and quality assessment of evidence

The methodological quality of included studies was assessed for risk of bias according the recommendations of the Cochrane Collaboration (Higgins & Green, 2011) by two reviewers (JO and JZ) independently, where studies were categorized into having 'low risk', 'high risk', or 'unclear risk' of bias. Any disagreements or discrepancies were resolved through discussion or by a third reviewer (GD).

Two review authors worked independently and in parallel to assess the full-text of included studies. The following data were extracted from each study using an EXCEL spreadsheet (Microsoft, Redmond, WA, USA): author, year, design, country, sample size, sex, mean age, control, intervention, type of protein supplementation, protein dosage (g/day) and duration (weeks), muscle mass outcomes, muscle strength outcomes and physical performance outcomes.

Each outcome extracted was categorized into either 'very low', 'low', 'moderate' or 'high' according to GRADE criteria (Ryan & Hill, 2003), that assessed the quality of evidence based on eight criteria: risk of bias, inconsistency, indirectness, imprecision, publication bias, large magnitude of effect, dose response and effect of all plausible confounding factors.

2.4. Data analysis

Only RCTs assessed to have low risk of bias were included in the meta-analysis. The random effects meta-analyses with pooled mean raw difference between intervention and control group at 12-week and 24-week follow-up were performed for each outcome separately using the methods of DerSimonian and Laird (DerSimonian & Laird, 1986). Outcomes that were measured on different scales (leg press) were pooled using standardized mean difference, more specifically Hedges g (Hedges, 1981). The between study heterogeneity was estimated using the Mantel-Haenszel method (Mantel & Haenszel, 1959).

The correlation between pre and post measures required for the inclusion of a crossover study was derived from the study that reported sufficient information (Payette et al., 2002; Tieland, Dirks et al., 2012). For one of the outcomes, leg press, this information was not available and the correlation of 0.8 was used. A bias due to small study effect was evaluated by visual inspection of funnel plots and Egger, Davey Smith, Schneider, and Minder (1997) regression asymmetry test (36) (Supplemental Material 2). All analyses were performed using Stata 15.1 (StataCorp, 2017). Sensitivity analyses were performed on the studies using Fried Frailty Criteria.

3. Results

3.1. Studies characteristics

A total of 14,646 articles were identified through the literature search. After removing duplicates (n = 9,512), 5,134 articles were identified of which 4,959 were excluded during title and abstract screening (Fig. 1). Following full-text review of 175 articles, eight studies comprising 503 participants were included for the qualitative analysis and six studies were included in meta-analysis.

The included studies were conducted in the United States of America, Canada, Europe and Asia covering Caucasian and Asian populations both community-dwelling and residents of aged care facilities. Seven studies were RCTs and one study was a crossover RCT (Table 1). Two duplicate studies conducted by Tieland et al were two separate arms of the same RCT (Tieland, Dirks et al., 2012; Tieland, van de Rest et al., 2012). General characteristics of the studies are summarized in Table 1. The average age ± standard deviation (SD) of the participants in the included studies ranged from 77 ± 1 to 89 ± 0.8, where 62.6% of the participants were female. Study sizes ranged from 20 to 100 participants and follow up duration ranged from eight weeks to 24 weeks. All participants were classified as frail according to a variety of frailty screening tools such as FFC (n = 213) (Dirks et al., 2017; Ikeda et al., 2016; Tieland, Dirks et al., 2012; Tieland, van de Rest et al., 2012), FWG (n = 87) (Kim & Lee, 2013), residential status in aged care facility and functional status (n = 100) (Fiatarone et al., 1994), and weight maintenance status (n = 83) (Payette et al., 2002). According to FFC, participants would be considered as pre-frail if they met one or two of the criteria and frail if they met three or more of the criteria proposed by Fried including unintentional weight loss, weakness, self-reported exhaustion, slow walking speed, and low physical activity (Fried et al., 2001). Similarly, FWG assessed eight domains of frailty such as mobility, muscle strength, nutritional intake, weight change, balance, endurance, fatigue and physical activity (Ferrucci et al., 2004). However, Kim and Lee (2013) focused on mobility and nutrition, two out of eight domains, as they were deemed to have strong association with the development of frailty. One study did not specify inclusion and exclusion criteria but was included as the study mentioned the recruitment of frail participants (Hankey, Summerbell & Wynne et al., 1993). With regards to nutritional status, one study specifically screened for low protein diet using Mini Nutritional

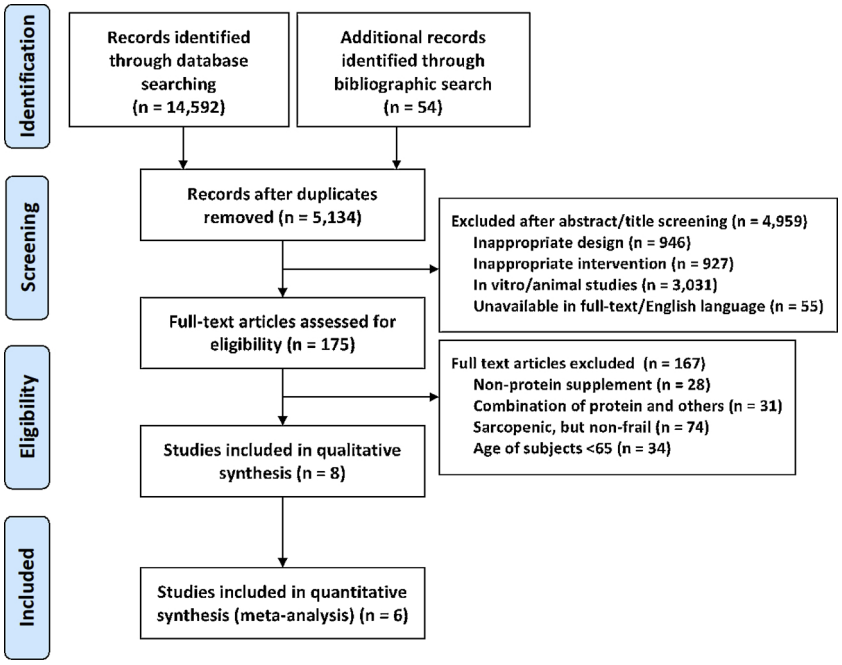

Fig. 1. - PRISMA Flowchart of the review process.

Assessment (MNA), where an MNA score lower than 24 indicates protein intake that is below recommended value (Kim & Lee, 2013; Xu, Tan, Zhang, Gui, & Yang, 2014). There was no record of baseline protein intake in other studies.

Three studies included exercise programs where both control and intervention groups received the same exercise programs, but only the intervention group received protein supplementation (Dirks et al., 2017; Ikeda et al., 2016; Tieland, Dirks et al., 2012). These studies showed significant benefit of protein supplementation in improving muscle mass, strength and function compared to the control group during the exercise program (Dirks et al., 2017; Ikeda et al., 2016; Tieland, Dirks et al., 2012). Participants randomly received a type of protein supplementation or placebo supplementation or activities. The dose and type of protein supplementation ranged from around 12 g/week to 32 g/day provided in the form of milk or soy-based protein, essential amino acid mixture, or commercially available nutritional drinks. The control treatments were normal hospital provision, placebo supplement containing no protein, maltodextrin capsules, or monthly visit with a small gift.

3.2. Quality of included studies and risk of bias

Three studies were at a high risk of allocation concealment bias (selection bias) and performance bias. Six of eight studies were at a high risk of detection bias as they recorded dietary intakes or physical functioning from the reports made by participants, or assessors were not blinded to the group allocations (Supplemental Fig. 2). None of the studies showed clear evidence of small study effect bias.

Six outcomes were assessed for quality of evidence through GRADEpro (Ryan & Hill, 2016), of which four outcomes were considered low and two were considered very low in certainty of evidence (Supplemental Fig. 3).

3.3. Main outcomes

The outcome measures investigated in this analysis included lean body mass, handgrip strength, leg press strength, leg extension strength, SPPB score, and gait velocity.

Three studies investigated change in lean body mass that was measured using dual X-ray absorptiometry (DXA) (Dirks et al., 2017; Tieland, van de Rest et al., 2012; Tieland, Dirks et al., 2012), two of which showed improvement following protein supplementation (Dirks et al., 2017; Tieland, van de Rest et al., 2012b). However, when summary measures at follow-up were used to compare the between group differences in a uniform manner for meta-analysis, none of the studies showed significant differences. Similarly, there was no overall difference between intervention and control group at 12-week or 24-week (Fig. 2) follow-up (mean difference at 12 weeks 1.17 kg, 95% CI: −1.97–4.3, p = 0.465; mean difference at 24 weeks 1.37 kg, 95% CI: −1.73–4.47, p = 0.386). The heterogeneity of this outcome was determined to be low (p = 0.547, I^2 = 0.0%).

In assessing the effect of protein supplementation on muscle strength, five studies measured handgrip strength (Dirks et al., 2017; Kim & Lee, 2013; Ikeda et al., 2016; Tieland, Dirks et al., 2012; Tieland, van de Rest et al., 2012), five studies measured leg press strength (Dirks et al., 2017; Fiatarone et al., 1994; Ikeda et al., 2016; Tieland, Dirks et al., 2012; Tieland, van de Rest et al., 2012;), and three studies measured leg extension strength (Dirks et al., 2017; Tieland, Dirks et al., 2012; Tieland, van de Rest et al., 2012). There was no statistical difference between intervention and control group at 12 weeks or 24 week for handgrip strength (mean difference at 12 weeks 0.15 kg, 95% CI: −0.95–1.24, p = 0.795; mean difference at 24 weeks 0.93 kg, 95% CI: −2.44–4.31, p = 0.587) (Fig. 3), leg press strength (mean standardized difference at 12 weeks 0.26 kg, 95% CI: −0.30–0.82, p = 0.370; mean difference at 24 weeks 2.79 kg, 95% CI: −11.25–16.84, p = 0.697) (Fig. 4**A and B**) or leg extension strength (mean difference at 12 weeks −3.68 kg, 95% CI: −12.72–5.36, p = 0.425; mean difference at 24 weeks −0.94 kg, 95% CI: −8.36–6.47, p = 0.803) (Fig. 4**C and D**). The heterogeneity of the studies included in the quantitative analysis was found to be low in handgrip strength and leg extension strength (p = 0.465, I^2 = 0.0% for handgrip strength and p = 0.230, I^2 = 31.9% for leg extension strength), but significant for leg press strength (p = 0.000, I^2 = 85.5%). One study conducted by Fiatarone et al. (1994) was not eligible for meta-analysis due to variation in the methods of measuring muscle strength where each individual limb strength was recorded separately. Inclusion of that study in this meta-analysis (had the methods been the same) would not alter the conclusion as no statistical difference

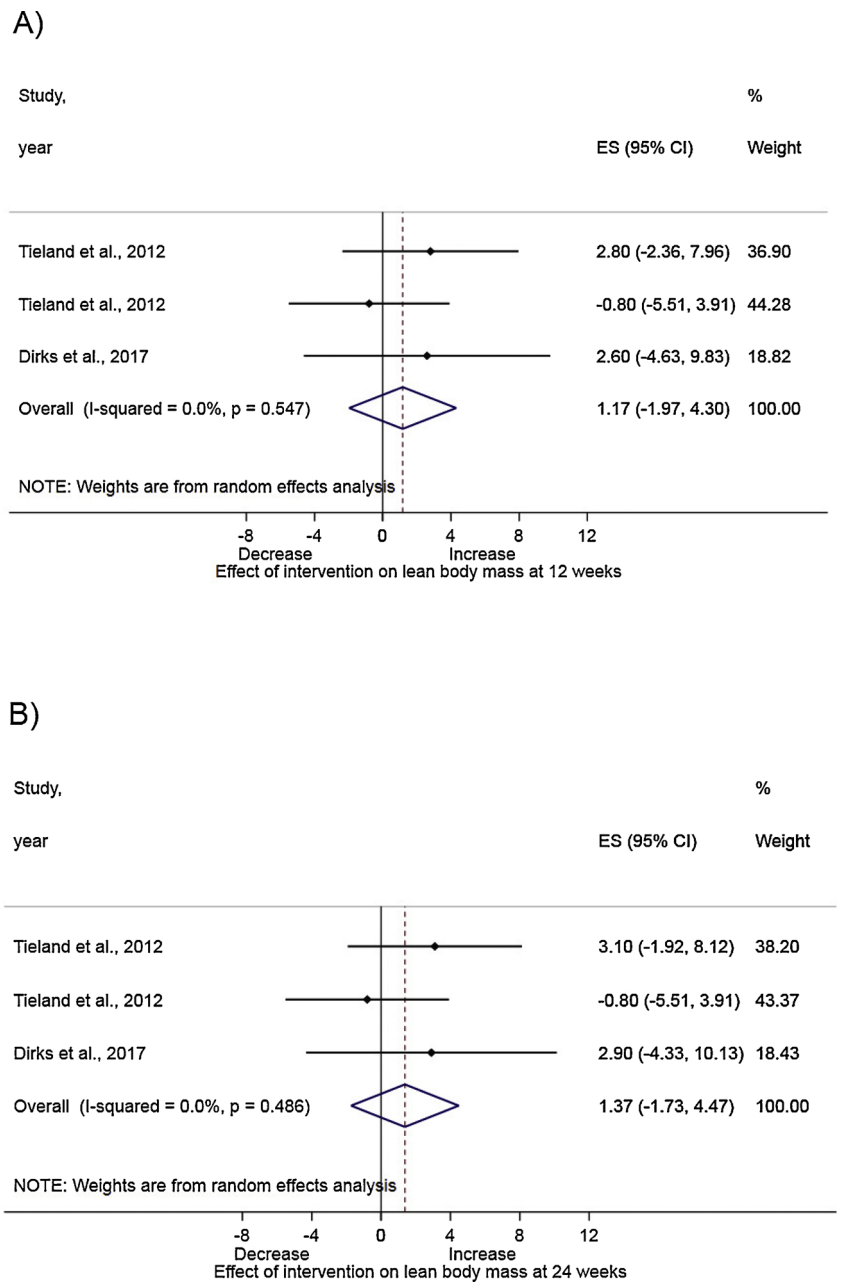

Fig. 2. – **A-** Forest plot showing no effect of protein supplementation on lean body mass at 12 weeks (kg).
B- Forest plot showing no effect of protein supplementation on lean body mass at 24 weeks (kg).

between control and intervention group was found.

The effect of protein supplementation on muscle function was assessed by performing SPPB in four studies (Dirks et al., 2017; Kim & Lee, 2013; Tieland, Dirks et al., 2012; Tieland, van de Rest et al., 2012), and gait velocity in four studies (Dirks et al., 2017; Fiatarone et al., 1994; Tieland, Dirks et al., 2012; Tieland, van de Rest et al., 2012. Fiatarone et al. (1994) was not eligible for the meta-analysis of physical function due to the use of different unit in measuring gait velocity. In this study, protein supplementation was not associated with improvement in gait velocity (Fiatarone et al., 1994). The included studies were assessed for heterogeneity, which was minimal for both outcomes ($p = 0.356$, $I^2 = 7.5\%$ for SPPB and $p = 0.374$, $I^2 = 0.0\%$ for gait velocity). Protein supplementation was found to have minimal impact in improving SPPB score at 12 weeks (mean difference 0.61, 95% CI −0.02–1.23, $p = 0.057$), but not at 24 weeks (mean difference 0.52, 95% CI: −0.94–1.98, $p = 0.482$) (Fig. 5**A and B**). No effect was found in gait velocity when comparing intervention and control groups at 12 weeks and 24 weeks (mean difference at 12 weeks −0.20 seconds (s), 95% CI: −0.95–0.55, $p = 0.606$; mean difference at 24 weeks 0.06 s, 95% CI: −0.67–0.80, $p = 0.863$) (Fig. 5**C and D**). The study conducted by Hankey et al. (1993), was not eligible for meta-analysis due to variation in the outcome measures included in the study. That study recruited 20 frail older subjects and reported the potential benefit of protein supplementation in this population (1993).

Sensitivity analysis was performed on studies using FFC, which showed no significant difference when compared with the pooled analysis (Dirks et al., 2017; Ikeda et al., 2016; Tieland, Dirks et al., 2012; Tieland, van de Rest et al., 2012).

4. Discussion

The purpose of this review was to assess the effect of protein

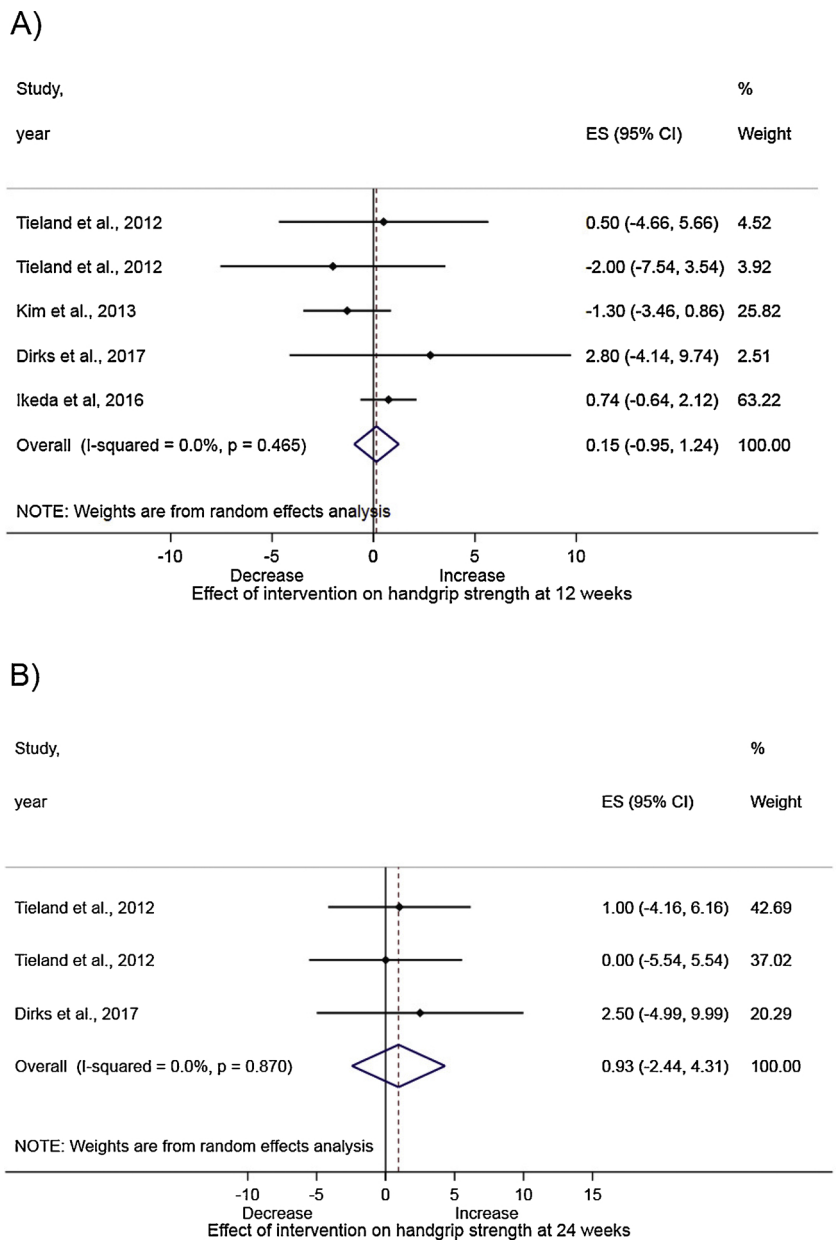

Fig. 3. – A- Forest plot showing no effect of protein supplementation on handgrip strength at 12 weeks (kg).
B- Forest plot showing no effect of protein supplementation on handgrip strength at 24 weeks (kg).

supplementation on muscle mass, strength and function in frail older people. Despite the borderline improvement in SPPB score, this meta-analysis demonstrates that protein supplementation does not have any significant benefit in improving lean body mass, leg strength, handgrip strength or gait velocity, which are considered as the major indicators of physical frailty.

Our findings had similarities with one previous meta-analysis (Tieland et al., 2017) but showed some discrepancies with other meta-analyses in this area (Katsanos, Kobayashi, Sheffield-Moore, Aarsland, & Wolfe, 2005; Komar, Schwingshackl, & Hoffmann, 2014), and these discrepancies could be due to differences in inclusion and exclusion criteria. Unlike, Tieland et al. (2017) and Xu et al. (2014), our study only included studies that recruited older individuals who were considered frail and that solely assessed the effect of protein supplementation. If combined intervention was present, we screened the methodology to ensure that both intervention and control groups received the same additional interventions, and that protein supplementation was the only variable being trialed. This allowed us to exclusively investigate the effect of protein supplementation on muscle mass, strength and function in frail older subjects. We also analyzed, pooled and standardized the original data from included studies.

There are many factors that could contribute to the outcome of this review and meta-analysis including the frailty screening tools used to recruit participants, amount and types of protein supplementation, and the duration of intervention. The RCTs included in this analysis screened their participants using FFC (Dirks et al., 2017; Ikeda et al., 2016; Tieland, Dirks et al., 2012; Tieland, van de Rest et al., 2012), FWG (Kim & Lee, 2013), residential status in an aged care facility (Fiatarone et al., 1994), and severity of involuntary weight loss within the last six months (Payette et al., 2002). The majority of included studies that defined frailty using specific and widely used screening tools such as FFC and FWG reported potential benefits of proteins supplementation on either muscle mass, strength or function (Dirks et al., 2017; Kim & Lee, 2013; Tieland, Dirks et al., 2012; Tieland, van de Rest et al., 2012). Two studies that did not use any specific screening tool showed no significant effect of protein supplementation (Fiatarone

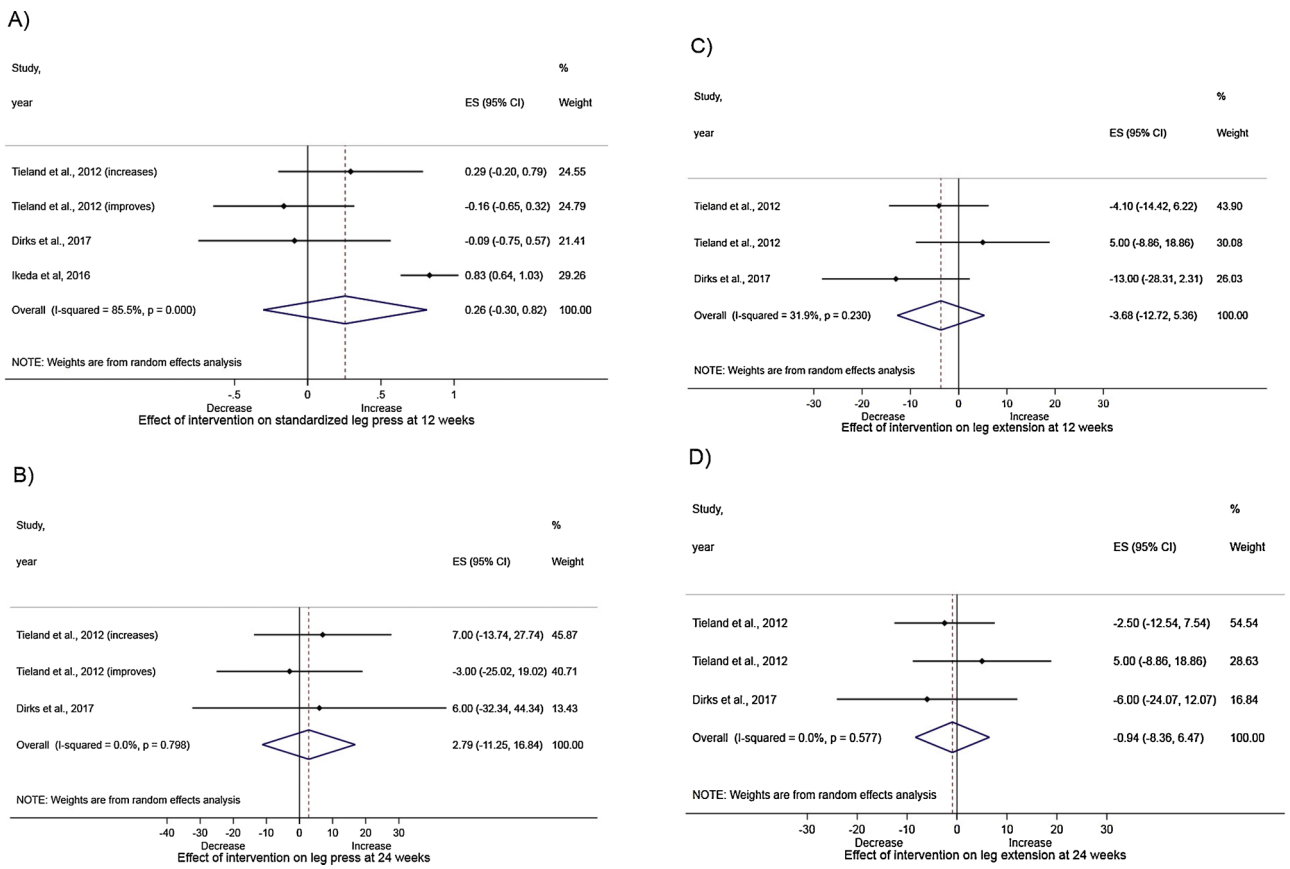

Fig. 4. – A- Forest plot showing no effect of protein supplementation on leg press at 12 weeks (kg).
B- Forest plot showing no effect of protein supplementation on leg press at 24 weeks (kg).
C- Forest plot showing no effect of protein supplementation on leg extension strength at 12 weeks (kg).
D- Forest plot of the effect of protein supplementation on leg extension strength at 24 weeks (kg) showing no difference between intervention and control group.

et al., 1994; Payette et al., 2002). Hence, eligibility criteria of participants may correlate with the outcome of studies. The use of specific frailty screening tools may be crucial in recruiting participants who were sufficiently compromised in functional and nutritional status, and who could gain more benefits from protein supplementation (Tieland et al., 2017).

The amount of dietary protein ranged from approximately 12 g/week (Ikeda et al., 2016) to 32 g/day (Payette et al., 2002), with the majority of included studies evaluated an amount of 30 g of protein per day (Hankey et al., 1993; Tieland, Dirks et al., 2012; Tieland, van de Rest et al., 2012). It is recommended that a minimum amount of 1.0 to 1.2 g/kg/day for individuals over 65 be required to ensure adequate nutritional intake (Bauer et al., 2013). The benefit of dietary intake is also maximized if taken over several meals in smaller amounts compared to single large bolus of protein (Rutherfurd, Fanning, Miller, & Moughan, 2015). Despite showing some significant improvement in two muscle strength indicators, leg press and knee extension, an intake of approximately 12 g of branched-chain amino acid (BCAA)/week may be inadequate to improve muscle strength as there was no significant benefits found in other strength assessments (Ikeda et al., 2016). However, having approximately 32 g of protein as dietary supplement per day still demonstrated no increase in muscle strength (Payette et al., 2002). The low number of included studies requires more RCTs to be conducted in order to confirm this finding.

In addition to the amount of protein supplementation, the type of protein may also have a role in determining the outcome of included studies. The types of protein supplements investigated in our meta-analysis included soy-based protein (Fiatarone et al., 1994), milk-based protein (Dirks et al., 2017; Payette et al., 2002; Tieland, Dirks et al., 2012; Tieland, van de Rest et al., 2012), BCAA supplement containing a mixture of EAAs (Ikeda et al., 2016), enteral nutrition containing high protein concentration (Kim & Lee, 2013), and a commercially available supplement with unspecified content (Hankey et al., 1993). The different sources of protein have different digestibility values, where milk protein concentrate that was provided in three studies (Dirks et al., 2017; Tieland, Dirks et al., 2012; Tieland, van de Rest et al., 2012) has lower digestibility value compared to soy-based protein (Baum & Wolfe, 2015). Digestibility value of protein refers to the rate of digestion and absorption of amino acids contained in dietary protein from the gut upon consumption (Churchward-Venne et al., 2014). EAA is also absorbed faster, resulting in more rapid increase in plasma amino acid concentrations compared to intact protein as found in milk protein (Churchward-Venne et al., 2014). However, a study testing the efficacy of varying protein-EAA doses found that the lower digestibility of milk-based did not have any impact in enhancing MPS (Churchward-Venne et al., 2014). Hence, the different types of protein supplementation being assessed in the included RCTs could have similar capacity in stimulating MPS and would not have significant influence on the outcome of included studies.

Another factor that may affect the outcome of studies is the duration of study. In the present analysis, the duration of included studies varies between eight to 24 weeks. Five out of eight studies were conducted over < 24 weeks, which may be inadequate to observe any changes in outcome measures. Considering the annual rate of loss of muscle in

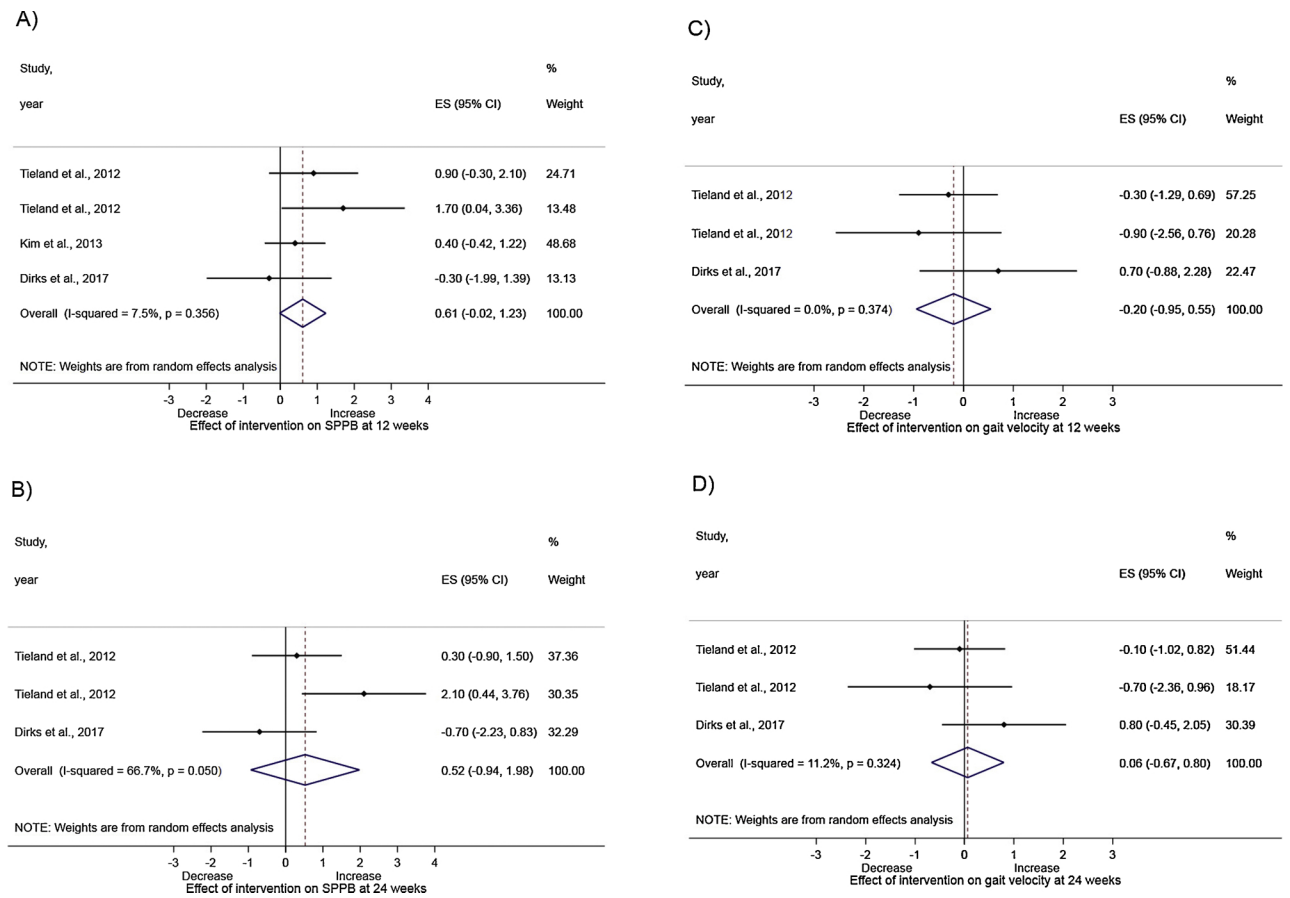

Fig. 5. – A- Forest plot showing minimal effect of protein supplementation on SPPB score at 12 weeks showing minimal improvement in intervention group.
B- Forest plot showing no effect of protein supplementation on SPPB score at 24 weeks.
C- Forest plot showing no effect of protein supplementation on gait velocity at 12 weeks (s).
D- Forest plot showing no effect of protein supplementation on gait velocity at 24 weeks (s).

adults is around 1% per year (Goodpaster et al., 2006), the amount of protein administered over < 24 weeks may be insufficient to allow any improvement in muscle mass, strength or function. Furthermore, the equipment used to assess outcomes may not detect any changes, that could possibly be small in studies lasting < 24 weeks, due to equipment error rate. Therefore, more studies that assess the effect of protein supplementation in frail older people over a longer period of time are required.

In this study, we investigated the effect of protein supplementation exclusively without any additional intervention such as exercise. Combination of exercise and protein supplementation seems to have greater impact in attenuating loss of muscle mass, strength and function in frail older people as shown by clinical trials that provided exercise program for both control and intervention groups (Dirks et al., 2017; Tieland, Dirks et al., 2012). Physical activity increases dietary protein uptake by stimulating skeletal muscle tissue, resulting in the augmentation of muscle protein synthesis rate (Pennings et al., 2011). The data suggests that by providing exercise program and protein supplementation simultaneously, the attenuation of the loss of muscle mass, strength and function in older people could be achieved. In frail older persons with physical or cognitive limitations, the addition of exercise programs to nutritional supplementation may not always be possible.

The main limitations of this study are the small number of studies and total sample size. This was due to the inclusion criteria that focused on frailty. Variations in the methodologies of included studies were also a limitation. An additional significant limitation is that baseline dietary intake is not universally measured. Thus negative conclusions drawn from our study regarding protein supplementation may have been confounded by background protein intake.

5. Conclusion

There is currently insufficient evidence to suggest the use of protein supplementation alone in pre-frail or frail older people attenuates the loss of muscle mass, strength and function. Further, well-designed RCTs in this area of research are necessary to better describe the role of protein supplementation in this population.

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Declaration of Competing Interest

The authors have no conflict of interest to declare.

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The author's responsibilities were as follows – JO and GD: designed the study; JO and JZ: conducted the study; SV: performed statistical analysis; JO: wrote paper; GD: had primary responsibilities for final content; all authors provided critical revision of the manuscript, read

and approved the final manuscript. None of the authors reported a conflict of interest related to the study.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.archger.2019.103938.

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