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Effects of leucine-rich protein supplements in older adults with sarcopenia: A systematic review and meta-analysis of randomized controlled trials

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ABSTRACT

Objectives: This study examined whether leucine-rich protein supplements improve muscle strength, mass, and performance in sarcopenic older adults.
Methods: We searched PubMed-Medline, Embase, and Cochrane Library databases for randomized controlled trials comparing leucine-rich protein supplements with a control intervention in sarcopenic older adults. A pairwise meta-analysis using a fixed-effects model was performed. The primary outcome of interest was muscle strength regardless of the measures used. Effect sizes were computed as standardized mean differences (SMDs) with 95% confidence intervals (CIs).
Results: Six randomized controlled trials including a total of 699 participants were retrieved. Leucine-rich protein supplements improved participants' overall muscle strength, mass, and performance compared to the control group (SMD=0.939; 95% CI, 0.440–1.438; $P<0.001$). As the primary outcome, muscle strength improved significantly in the leucine group (SMD=0.794; 95% CI, 0.104–1.485; $P=0.024$).
Conclusion: Leucine-rich protein supplements improve muscle strength in sarcopenic older adults. They may be suggested in nutritional treatment of sarcopenia.

1. Introduction

The treatment of choice for sarcopenia is still resistance exercise with nutritional supplementation because no pharmacological agents to treat sarcopenia have not become available yet (Osuka et al., 2019; Kwak and Kwon, 2019). While resistance exercise should be added to maximize the effects of the nutritional intervention (Yoshimura et al., 2017), nutritional interventions remain the most promising treatment and prevention strategies for many older adults who are unable to exercise (Martínez-Arnau et al., 2020; Lee et al., 2021; Jang et al., 2021). Supplementation with the branched-chain amino acid, leucine, or leucine-enriched protein (whey/casein protein) is one of the most common interventions for treating sarcopenia in older individuals (Yoshimura et al., 2019; Martínez-Arnau et al., 2020; Kim et al., 2012; Bauer et al., 2015; Amasene et al., 2019; Baek et al., 2021).

Leucine is an essential branched-chain amino acid that regulates muscle function partly through the mammalian target of rapamycin (mTOR) pathway (De Bandt, 2016). It has been shown to modify protein turnover in skeletal muscles by decreasing proteolysis and increasing protein synthesis (Casperson et al., 2012; Rieu et al., 2006; Wall and van Loon, 2013). Furthermore, it can increase skeletal muscle glucose absorption and induce insulin release in pancreatic cells, which is an important anabolic signal in skeletal muscle (van Loon et al., 2003). Therefore, dietary supplementation with leucine has been investigated as one strategy to increase muscle protein synthesis, and is a promising approach for treating sarcopenia (Martínez-Arnau et al., 2019).

However, recent studies confirming the effects of nutritional interventions with leucine were not performed specifically in subjects with sarcopenia (Kim et al., 2021) or used a combination of various nutrients including leucine (Lin et al., 2021). Therefore, we conducted a meta-analysis to evaluate whether leucine-rich protein supplement can improve muscle quality and quantity in sarcopenic older adults. We

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compared muscle strength, mass, and physical performance between leucine-rich protein supplementation and control groups. We hypothesized that leucine-rich protein supplementation would yield improvements in muscle quality and quantity compared to untreated controls in patients with sarcopenia.

2. Methods

2.1. Search strategy

This meta-analysis was conducted according to the updated Preferred Reporting Items for Systematic Review and Meta-Analysis Protocols (PRISMA-P) guidelines. The PubMed-Medline, Embase, and Cochrane Library databases were searched in January 2022 using the following key terms: (sarcopenia OR presarcopenia OR frailty OR pre-frailty OR muscle weakness) AND (leucine OR isoleucine OR valine OR "Branched-Chain Amino Acid") AND (muscle mass OR strength OR performance OR gait OR walking). An overview of the search strategy is presented in online supplementary A.

2.2. Study selection criteria

All identified records were saved in EndNote (ver. 20; Thomson Reuters Corp., Toronto, ON, Canada). Two independent reviewers (SYL, JYL) first screened the titles and abstracts to ensure that the studies were relevant. We included all randomized controlled trials (RCTs) that compared leucine-rich protein supplements with a control intervention in sarcopenic older adults. There were no limitations on the surgical procedure or language of the report. The inclusion criteria were RCTs and controlled interventions with leucine-rich protein supplementation in sarcopenic older adults. We included nutritional interventions with isoleucine, which is an isomer of leucine, valine, and branched-chain or essential amino acids to increase the sensitivity of the search. Reviews, basic science articles, comments, letters, and protocols were excluded. When studies involving the same cohort of patients were identified, only the latest update was included in the analysis.

2.3. Outcome measures and data extraction

The primary outcome of interest was muscle strength regardless of the measures used. The various measurements had their own variables (handgrip strength, chair stand test, knee extensor strength, and arm curl test). The secondary outcomes were other variables related to sarcopenia as follows: muscle mass (skeletal muscle mass index measured using bioimpedance analysis or dual-energy X-ray absorptiometry) and physical performance (Short Physical Performance Battery, gait speed, physical fitness test, balance score, and so forth).

2.4. Quality assessment and publication bias

Two authors (SYL, JYL) independently assessed the quality of studies included using the criteria of the Cochrane Handbook for Systematic Reviews of Interventions (Higgins and Green, 2011), which include the following seven items: random sequence generation; allocation concealment; blinding of participants and personnel; blinding to outcome data; incomplete outcome data addressed; selective reporting; and other bias. We assessed publication bias using Begg's funnel plot and Egger's test.

2.5. Statistical analysis

Effect sizes were computed as standardized mean differences (SMDs). Thus, the pre/post-test differences in all outcomes were analyzed. SMDs were computed separately for all control and treatment groups in each study. Heterogeneity among comparable studies was tested using the χ^2 and I^2 tests. Values of $P>0.1$ and $I^2<50\%$ were taken to indicate statistical significance. As significant heterogeneity was apparent among the six studies ($P<0.001$ and $I^2=83.3\%$), we used a random-effects meta-analysis model to measure the pooled effect size of the included studies. For each analysis according to outcome parameters, we also used random-effects model to analyze the effects of leucine-rich protein supplements on muscle strength ($P<0.001$ and $I^2=93.8\%$), muscle mass ($P<0.001$ and $I^2=97.1\%$), and physical performance ($P<0.001$ and $I^2=92.5\%$). All analyses were performed using Comprehensive Meta-Analysis Software (version 3.3; Biostat, Englewood, NJ, USA). Our study was exempted from Institutional Review Board review because we did not deal directly with any human subjects. This study conformed to all PRISMA guidelines and reports the required information accordingly (see online supplementary B: PRISMA checklist).

3. Results

3.1. Description of included studies

After database searches and full-text review, six studies met all of the quality-related inclusion criteria (Yoshimura et al., 2019; Martínez-Arnau et al., 2020; Kim et al., 2012; Bauer et al., 2015; Amasene et al., 2019; Rondanelli et al., 2020). Fig. 1 presents the excluded and included studies and Table 1 summarizes the characteristics of the included studies. The same six RCTs (performed from 2008 to 2018) fulfilled the inclusion criteria for quantitative analysis. All six studies defined sarcopenia in elderly subjects using established diagnostic criteria, such as those of the European Working Group on Sarcopenia in Older People (EWGSOP), Asian Working Group for Sarcopenia (AWGS). In only one study (Yoshimura et al., 2019), subjects were defined as post-stroke older patients with sarcopenia rather than primary sarcopenia. The amount of leucine intake was 3 g per day in 2 studies (Amasene et al., 2019; Kim et al., 2012) and 6 g per day in the remaining 4 studies. Other nutrients (lactose, maltodextrin, vitamin D, and isocaloric product) were given to the control group in four studies (Martínez-Arnau et al., 2020; Amasene et al., 2019; Bauer et al., 2015; Rondanelli et al., 2020), while the control group received a non-nutritional treatment, such as health education, in two studies (Yoshimura et al., 2019; Kim et al., 2012). The duration of nutritional intervention ranged from 4 to 13 weeks.

3.2. Results

Six randomized controlled trials including a total of 699 participants were retrieved. Leucine-rich protein supplements improved participants' overall muscle strength, mass, and performance compared to the control group (SMD=0.939; 95% confidence interval, 0.440–1.438; $P<0.001$) (Fig. 2). As the primary outcome, muscle strength improved significantly in the leucine group (SMD=0.794; 95% CI, 0.104–1.485; $P=0.024$) (Fig. 3A). In terms of secondary outcomes, muscle mass (SMD=0.763; 95% CI, -0.353–1.880; $P=0.180$) and physical performance (SMD=0.788; 95% CI, -0.010–1.586; $P=0.053$) tended to improve in the leucine group, although these trends were not significant (Fig. 3B and 3C).

In terms of safety issues, no serious adverse events were reported in all six papers. Nutritional supplementation was well tolerated (no gastro-intestinal intolerance) although a few subjects refused to take supplements according to their taste preferences (Rondanelli et al., 2020). Also, in one study, one minor adverse event was reported with protein supplementation regarding itchy throat and difficulties to inhale in one subject (Amasene et al., 2019).

3.3. Quality assessment and publication bias

In terms of methodological quality, all subjects were randomized using established allocation sequences, and the investigators and research assistants were blinded to the group allocation. However, it is unclear whether all trials met all quality assessment criteria (Fig. 4).

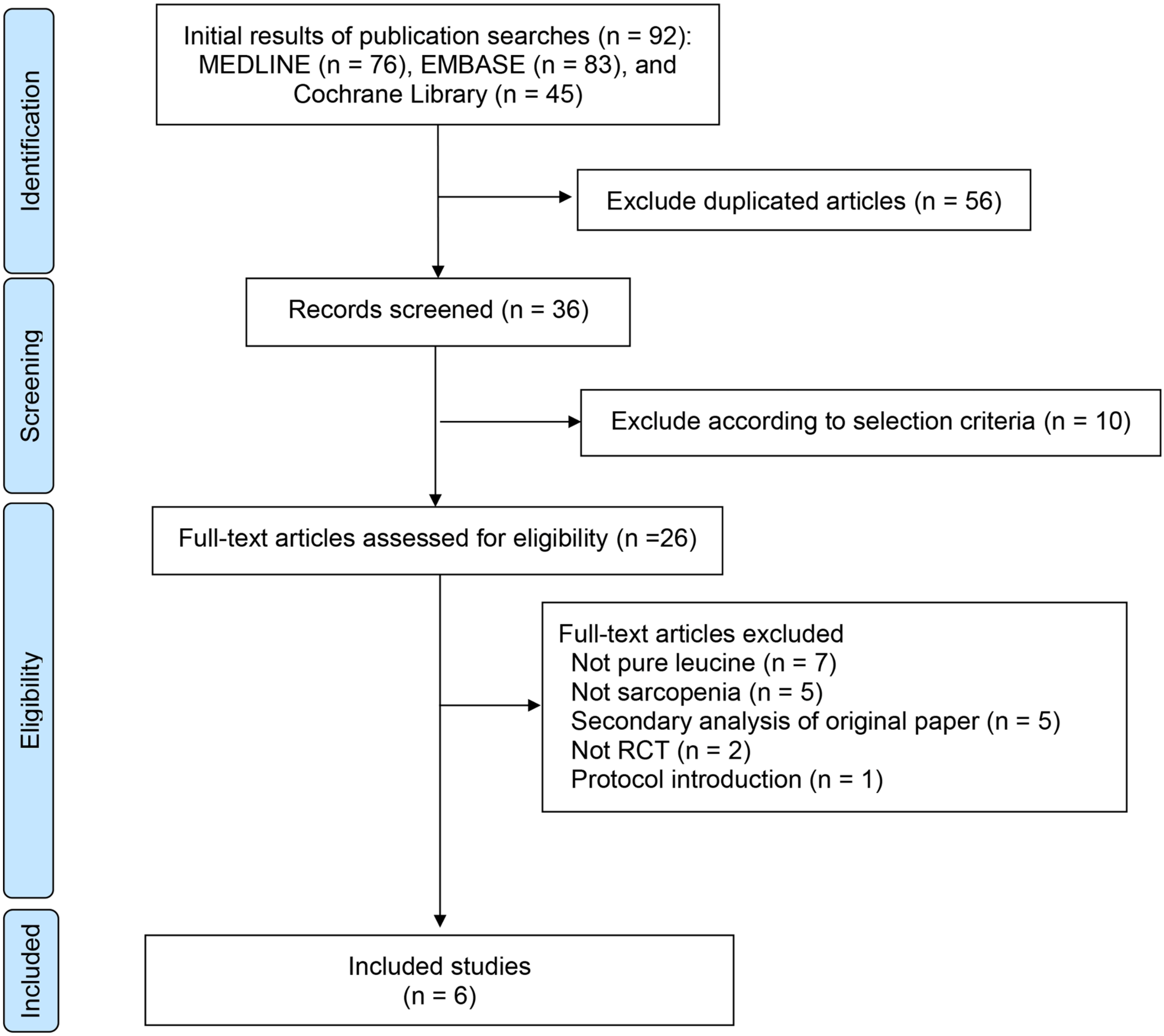

Fig. 1. A Preferred Reporting Items for Systematic Review and Meta-analysis flow diagram detailing our clinical study selection process.

Publication bias was not evident; the Begg's funnel plot was symmetrical (online supplementary C) and the bias was not significant according to Egger test of the six trials (P=0.768).

4. Discussion

In this meta-analysis to evaluate the effectiveness of leucine-rich protein supplementation in sarcopenic older adults, leucine-rich protein was suggested to improve muscle strength. Although muscle mass and physical performance tended to improve, these trends were not significant. To the best of our knowledge, this is the first meta-analysis investigating the effects of leucine-rich protein supplementation in sarcopenic older adults.

A combination of protein supplementation and resistance training was reported to have positive effects on body composition, muscle mass and strength, and physical function in elderly people (Liao et al., 2017). By contrast, Thomas *et al.* suggested that protein or essential amino acid supplementation did not significantly augment the effects of progressive resistance exercise training in older adults (Thomas et al., 2016). It cannot be denied that resistance exercise is the most important factor in the treatment of sarcopenia in older adults. However, it is challenging for older adults to maintain resistance exercise continuously, and it is unrealistic to recommend only exercise to frail and sedentary older adults. Therefore, adequate protein supplementation plays another role in the maintenance of function in older people (Morley, 2012), and nutritional intervention is an indispensable treatment option for the elderly with sarcopenia (Park et al., 2021).

Leucine is a key regulator of muscle protein synthesis, acting via the mTOR pathway (Ge et al., 2009), and consumption of leucine increases muscle protein synthesis and attenuates muscle soreness after exercise (Kato et al., 2015). A protein/amino acid source containing around 1.8–2.0 g leucine is sufficient to activate the post-exercise "leucine trigger," while under rested conditions, a higher dose may be required in older adults (Reidy and Rasmussen, 2016). One study suggested that the supplementation of 21 g of a leucine-enriched whey protein increases muscle protein synthesis rates in both sarcopenic and healthy older men and the rates are not lower in sarcopenic older men compared to healthy older men (Kramer et al., 2017).

Table 1
Characteristics of the included individual studies

Study	Study period	Region	Subject characteristics	Sarcopenia definition	Intervention group	Leucine dose (per day)	Control group	Common treatment in both groups	Duration	F/U period	No. of participants Intervention	Control	Outcomes Primary	Secondary
Martínez-Arnau et al. (2020)	July 2018	Spain	Aged 65 and over, living in nursing homes, able to walk 6 m	EWGSOP	Daily intake of leucine (6 g/ day)	6 g	Lactose, 6 g/ day	(-)	13 wks	13 wks	23	19	Sarcopenia and respiratory muscle function	Geriatric evaluation scales, blood analytical parameters and inflammatory markers
Rondanelli et al. (2020)	May 2017- Dec 2018	Italy	old adults (age ≥ 65 years) candidates for inpatient rehabilitation	EWGSOP	20 g of whey proteins, 2.8 g of leucine, 9 g of carbohydrates, 3 g of fat, 800 IU of vitamin D, and a mixture of vitamins, minerals (calcium, 500 mg), and fibres, twice daily	5.6 g	Iso-caloric control product	individualized, moderate-level physical fitness and muscle mass promoting program	4-8 wks	4-8 wks (at discharge)	64	63	Gait speed	chair-stand test, TUG, SPPB, handgrip strength, Tinetti scale, Barthel Index, skeletal muscle mass index
Amasene et al. (2019)	Sep 2017 - Jul 2018	Spain	Hospitalized patients older than 70 years old	EWGSOP	20 g of whey protein isolate enriched with 3 g of leucine	3 g	Maltodextrin 23g	Supervised resistance training program	12 wks	12 wks	15	13	Physical function test, body strength, aerobic capacity, SPPB	MNA, body composition, biochemical parameters
Yoshimura et al. (2019)	Sep 2014 - Apr 2017	Japan	Post-stroke older patients with sarcopenia	AWGS	3 g of leucine 40% enriched essential amino acids and 9.7 g of carbohydrate / day	3 g	None	Post-stroke rehabilitation program	8 wks	8 wks	21	23	FIM motor domain	skeletal muscle mass index, handgrip strength
Bauer et al. (2015)	Jun 2010 – May 2013	6 European countries	Sarcopenic primarily independent-living older adults	Ref from a European study	20 g whey protein, 3 g total leucine, 9 g carbohydrates, 3 g fat, 800 IU vitamin D, and a mixture of vitamins, minerals, and fibers (twice daily)	6 g	Iso-caloric control product	(-)	13 wks	13 wks	184	196	Handgrip strength and SPPB score	chair-stand test, gait speed, balance score, and appendicular muscle mass
Kim et al. (2012)	Oct – Nov 2008	Japan	Women aged 75 and older	Refs from two Asian studies	3 g of a leucine-rich essential amino acid mixture twice a day	6 g	Health education	(-)	3 mo	3 mo	39	39	Body composition, physical fitness tests	

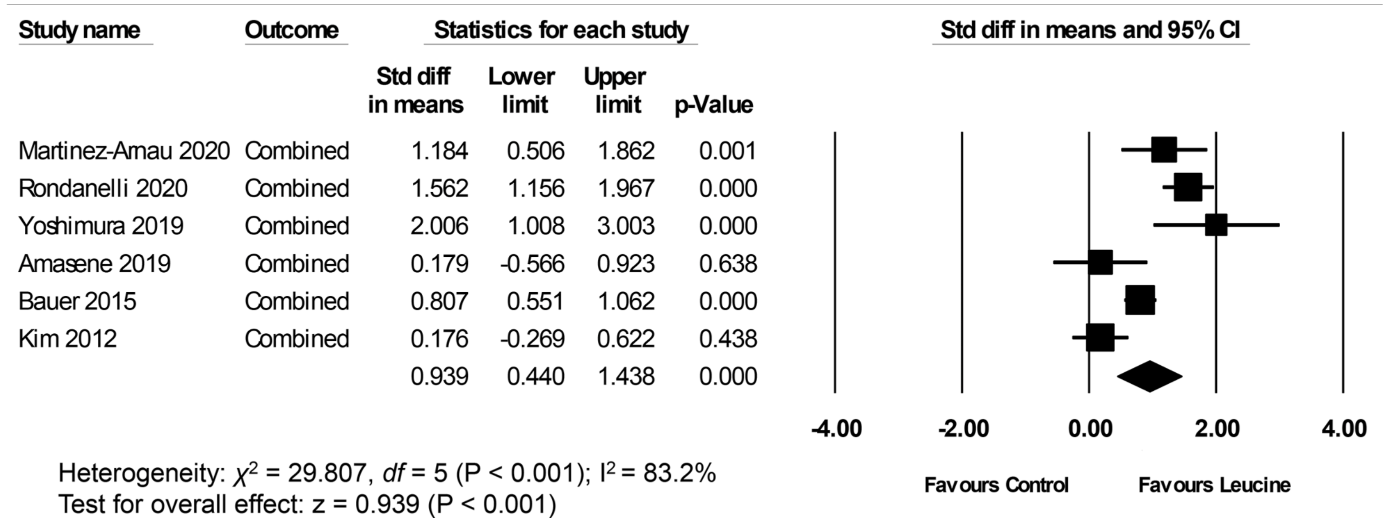

Fig. 2. Forest plot of the effects of leucine-rich protein supplementation in patients with sarcopenia determined by random-effects meta-analysis. Effect sizes are indicated as Hedges' g SMD and 95% CI.

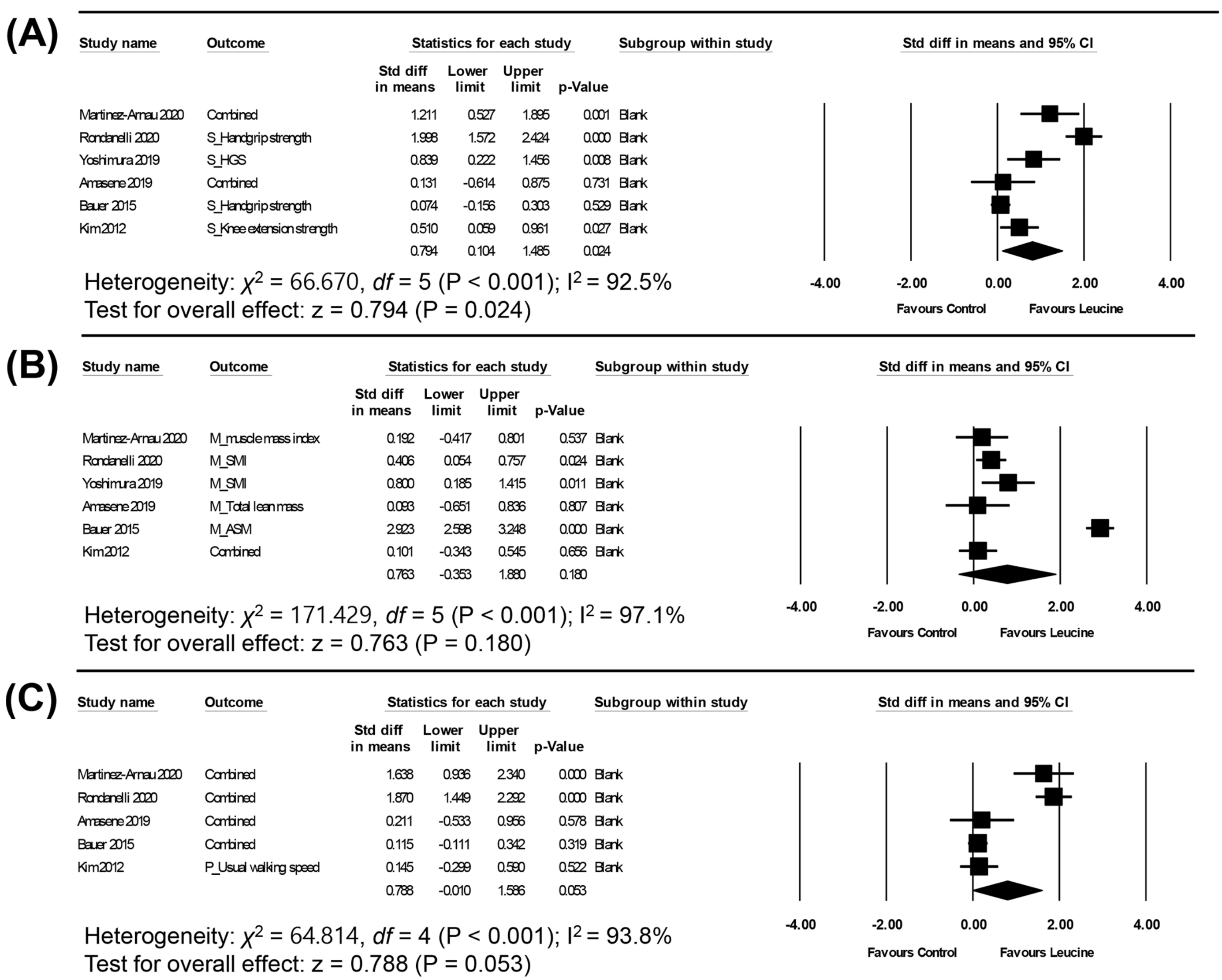

Fig. 3. Forest plots of the effects of trial-level characteristics of leucine-rich protein supplementation in patients with sarcopenia analyzed as outcome variables: (A) muscle strength, (B) skeletal muscle mass, and (C) physical performance. Effect sizes are shown as Hedges' g SMD with 95% CI. *Muscle mass, appendicular muscle mass, and leg muscle mass; †Physical function test, aerobic capacity, and SPPB; ‡SPPB, gait speed, and balance score.

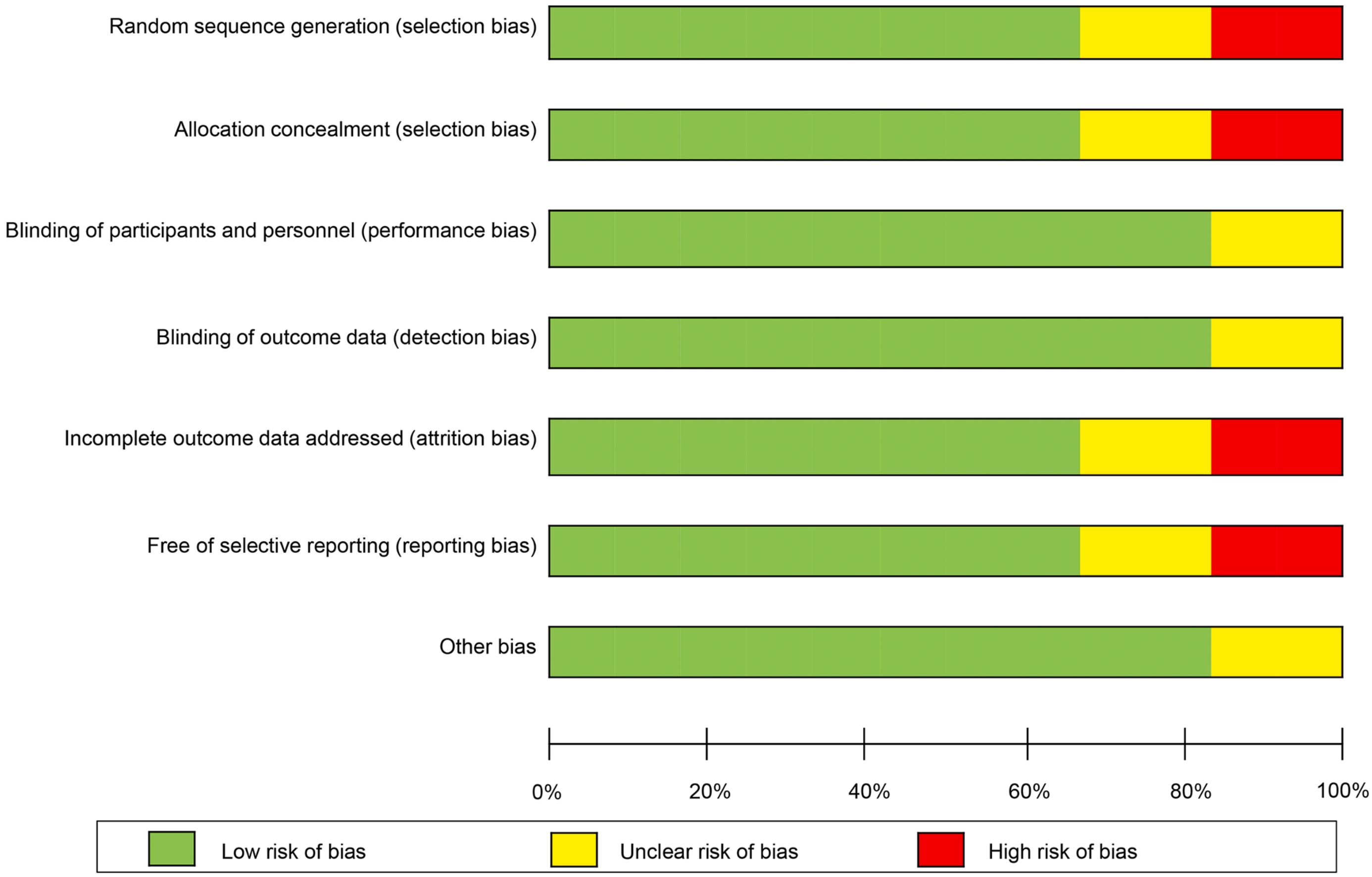

Fig. 4. Summary of bias risk for the randomized controlled trials included in the meta-analysis.

Leucine or leucine-enriched protein supplementation may also have other beneficial effects on cognitive function (Rondanelli et al., 2016), depressive mood (van de Bool et al., 2017), and quality of life (Bauer et al., 2015), which support the use of this intervention in geriatric populations. Thus, leucine-enriched nutritional supplements can preserve muscle mass and strength in older sarcopenic adults.

Does leucine supplementation have a dose-response effect on sarcopenia? To confirm this, we analyzed the six papers included in this meta-analysis by dividing them into a low dose group (3g) and a high dose group (5.6g or 6g) according to the daily leucine supplementation amount. Two papers were included in the low-dose group (Amasene et al., 2019; Kim et al., 2012), and the remaining four papers were all in the high-dose group. As a result of the analysis, the effect size (SMD) of the low dose group was 1.061 (95% CI -0.728 to 2.850) and that of the high dose group was 0.922 (95% CI 0.367 to 1.476), and no statistically significant difference was confirmed. If more related papers are published in the future, it is expected that the evidence for a more certain dose-response will be revealed.

Several interventions showed an improvement in lean mass after leucine supplementation co-administered with vitamin D as part of a nutritional intervention (Martínez-Arnau et al., 2020; Rondanelli et al., 2020). Vitamin D plays a vital role in normal muscle function, and low serum vitamin D level is associated with increased risk of falls and proximal weakness (Spira et al., 2019). Therefore, leucine and vitamin D supplementation must be taken into account as two of the most important factors in nutritional interventions for improving physical function in older adults.

Muscle mass did not increase significantly, while muscle strength improved in the leucine group in this meta-analysis. These findings were consistent with previous studies showing that protein supplementation enhanced physical performance in frail elderly adults without increasing skeletal muscle mass (Tieland et al., 2012; Bonnefoy et al., 2003). Changes in muscle strength and/or physical performance are usually seen before quantitative changes in skeletal muscle mass become apparent (Goodpaster et al., 2006). One meta-analysis of interventions for sarcopenia similarly reported that nutritional intervention was effective for improving knee extension strength, but there was no significant effect on appendicular skeletal muscle mass (Yoshimura et al., 2017).

This may result from the absence of linear relations among skeletal muscle mass, strength, and physical performance (Visser et al., 2000). Increases in neuromuscular activity and/or skeletal muscle quality may be responsible for such improvements in physical performance in the protein supplementation group before increments in muscle mass (Clark and Manini, 2008). However, Stragier *et al.* suggested that leucine-enriched protein supplementation did not influence neuromuscular adaptations to strength training in older adults (Stragier et al., 2016). Therefore, further in-depth studies on the mechanism by which leucine-rich nutritional intervention is effective for improving muscle strength in older adults with sarcopenia are warranted.

Our meta-analysis had several limitations. First, we included only six reports because there have been few studies comparing leucine-rich protein supplementation with control interventions in patients with sarcopenia. To overcome this limitation, we included various types of leucine supplement, different control group settings, and several outcome variables related to sarcopenia, which inevitably increased the heterogeneity of this study. In addition, it is necessary to distinguish between the effects of men and those of women in sarcopenia intervention. Five of the six papers recruited both male and female subjects, but did not present the results of separating men and women. Therefore, further large-scale, well-designed, and adequately powered RCTs are needed to raise the level of evidence. Second, we analyzed the effect sizes of several outcomes of individual studies in a pooled or overall manner. These findings have limited clinical usefulness and should be interpreted with caution. As the relevance of each outcome variable differed, the lack of weighting represented another limitation of the study. Third, as the included studies had limited follow-up periods (up to 3 months), we could not confirm the long-term effects of leucine-rich protein supplementation. Finally, there was publication bias as no unpublished studies were included in the analysis and all were written in English. However, an Egger test of the six trials showed that the bias was not significant.

5. Conclusion

Leucine-rich protein supplementation improves muscle strength in sarcopenic older adults and can be suggested as a nutritional treatment for sarcopenia.

Online supplementary material
Supplementary A: search strategy
Supplementary B: PRISMA checklist
Supplementary C: Begg's funnel plot

CRediT authorship contribution statement

Sang Yoon Lee: Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft, Visualization. **Hyun Jeong Lee:** Conceptualization, Methodology. **Jae-Young Lim:** Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft, Visualization.

Declaration of Competing Interest

None.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.archger.2022.104758.

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