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REVIEW ARTICLE

# Effect of supplementation with leucine alone, with other nutrients or with physical exercise in older people with sarcopenia: a systematic review

**Enrique Conde Maldonado**[a], **Diego Marqués-Jiménez**[a,b], **Patricia Casas-Agustench**[a,c,*], **Anna Bach-Faig**[d,e,*]

[a] *Facultad de Ciencias de la Salud, Universitat Oberta de Catalunya (UOC), Barcelona, Spain*
[b] *Deportivo Alavés (SAD) (Academia), Vitoria-Gasteiz, Spain*
[c] *School of Health Professions, Faculty of Health, University of Plymouth, Plymouth, UK*
[d] *Grupo de Investigación FoodLab (2017SGR 83), Facultad de Ciencias de la Salud, Universitat Oberta de Catalunya, Barcelona, Spain*
[e] *Vocalía de Alimentación y Nutrición, Colegio Oficial de Farmacéuticos de Barcelona, Barcelona, Spain*

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**KEYWORDS**
Sarcopenia;
Leucine;
Supplementation;
Physical exercise;
Aging;
Older people

**Abstract**
*Background:* Older adults are at a greater risk of developing sarcopenia as a result of reduced mobility, malnutrition, dietary changes and certain diseases. There are no systematic reviews in the literature analysing the effects of supplementation with leucine alone or as part of a supplement, and with or without physical exercise in older people with sarcopenia. We aimed to systematically review the evidence in intervention studies on the effects of supplementation with leucine, either alone, combined with other supplements, or combined with other supplements and physical exercise in older people with sarcopenia.
*Materials and methods:* Literature searches related to the topic were conducted in three databases (Pubmed/Medline, Cochrane and SciELO) looking for articles published prior to December 2020. This review includes intervention studies in older adults over 60 years of age with a history of sarcopenia where researchers reported on the effects of leucine supplementation, with or without physical exercise, related to the disease's treatments or outcomes.
*Results:* The systematic review identified three intervention studies examining the effect of leucine without physical exercise, one on leucine with physical exercise, seven on leucine paired with another nutrient and without physical exercise, and twelve on leucine paired with another nutrient and physical exercise. The results revealed that leucine supplementation alone and without physical exercise did not improve markers of sarcopenia, whereas interventions pairing leucine with supplements, particularly leucine-enriched protein supplements, are a promising treatment for the improvement of sarcopenic markers, whether with or without physical exercise.

* Corresponding author.
*E-mail addresses:* pcasasag@uoc.edu (P. Casas-Agustench), abachf@uoc.edu (A. Bach-Faig).

*Conclusions:* Leucine supplementation, specifically paired with protein supplements, both with and without physical exercise, was found to be an effective dietary intervention for the improvement of sarcopenia. Further dietary interventions are necessary to calculate effective dosage quantities for both leucine and nutrient supplementation as an integral part of the treatment.


**PALABRAS CLAVE**
Sarcopenia;
Leucina;
Suplementación;
Ejercicio físico;
Envejecimiento;
Personas adultas mayores

**Efecto de la suplementación con leucina sola, junto con otro nutriente o con ejercicio físico en personas adultas mayores con sarcopenia: una revisión sistemática**

**Resumen**
*Introducción:* Las personas adultas mayores tienen un mayor riesgo de desarrollar sarcopenia debido a la movilidad reducida, la desnutrición, los cambios en la dieta y determinadas enfermedades. Actualmente, no existen revisiones sistemáticas que analicen los efectos de la suplementación con leucina sola o como parte de un suplemento, ya sea con o sin la práctica de ejercicio físico en personas adultas mayores con sarcopenia. Nuestro objetivo es revisar sistemáticamente la evidencia en los estudios de intervención sobre el efecto de la suplementación con leucina, ya sea sola, junto con otro suplemento y con o sin ejercicio físico en personas adultas mayores con sarcopenia.
*Materiales y métodos:* Se realizaron búsquedas bibliográficas relacionadas con el tema en tres bases de datos (Pubmed/Medline, Cochrane y SciELO) buscando artículos publicados antes de diciembre de 2020. Esta revisión incluye estudios de intervención en personas adultas mayores de 60 años con antecedentes de sarcopenia donde los investigadores informaron sobre el efecto de la suplementación con leucina, con o sin ejercicio físico, en relación con los tratamientos o resultados de la enfermedad.
*Resultados:* La revisión sistemática identificó tres estudios de intervención que examinaron el efecto de la leucina sin ejercicio físico, uno de leucina con ejercicio físico, siete de leucina combinada con otro nutriente y sin ejercicio físico, y doce de leucina combinada con otro nutriente y ejercicio físico. Los resultados encontraron que la suplementación con leucina sola y sin ejercicio físico no mejoró los marcadores de sarcopenia, mientras que las intervenciones que combinan leucina con suplementos, particularmente suplementos proteicos enriquecidos con leucina, son un tratamiento prometedor para la mejora de los marcadores de sarcopenia, ya sean con o sin ejercicio físico.
*Conclusiones:* Se observó que la suplementación con leucina, específicamente combinada con suplementos de proteínas, con y sin ejercicio físico, es una intervención dietética eficaz para la mejora de la sarcopenia. Se necesitan más intervenciones dietéticas para calcular las cantidades de dosis efectivas tanto para la leucina como para la suplementación de nutrientes como parte integral del tratamiento.


## Introduction

Normal ageing in humans is one of the main risk factors for the functional decline of the body, as well as health in general, resulting in greater susceptibility to diseases. Certain factors are inherent to skeletal muscle function such as chronic low-grade inflammation,[1] decreased numbers of satellite cells and functional motor units,[2] mitochondrial dysfunction,[3] decreased levels of anabolic hormones[4] and loss of or deficiency in proteostasis,[5] causing numerous changes in body composition as muscles weaken and body fat levels increase. These changes result in what is called sarcopenia, a term coined by Rosenberg in 1989.[6] Sarcopenia presents as a muscle-wasting syndrome characterised by widespread, progressive loss of muscle mass and/or quality, muscle strength and/or physical ability occurring during ageing.[7] Therefore, sarcopenia can contribute to a higher risk of falls and fractures, mobility disorders and functional decline (gait speed, time taken to sit down, get up from a chair, climb stairs, etc.). This causes an increase in dependency; a decrease in quality of life; a higher risk of dysfunction; increased economic costs for healthcare and health maintenance; and, finally, a higher risk of death.[7]

The European Working Group on Sarcopenia in Older People (EWGSOP) was responsible for recognising sarcopenia as a geriatric syndrome. This working group established poor muscle function characterised by low muscle strength and reduced muscle mass as the main criteria for the clinical diagnosis thereof.[7] In 2019, the EWGSOP, in a new meeting to arrive at a consensus on the diagnosis of sarcopenia (EWG-

SOP2), updated the guidelines for defining sarcopenia and offered an operational definition that identifies and prioritises a decrease in muscle strength as the main parameter, confirmed by low muscle quality or quantity and considered serious due to poor physical fitness.[8]

Age is the main determining factor in an increase in sarcopenia and is directly proportional to the passage of time.[9] Loss of skeletal muscle mass starts gradually at age 50 and accelerates after age 70.[9] Its prevalence is approximately 5%–13% in people 60–70 years of age and as high as 50% among 80-year-old individuals.[10] Furthermore, loss of functional capacity in older adults with sarcopenia, which may entail situations of immobility, instability and intellectual decline, and is identified as a predictor of disability, hospitalisation and death, may be as high as 3% yearly as of 60 years of age.[10] Similarly, age-associated skeletal muscle loss is due to a disruption in skeletal muscle protein turnover[11] caused primarily by a lack of exercise and protein malnutrition, both of these being the primary causes of sarcopenia.[12]

Sedentary lifestyle and eating habits can affect an older adult's musculoskeletal conditions. Exercise, especially strength training, and nutritional interventions are the most commonly used strategies for mitigating this loss of muscle mass and muscle strength, in particular through improvements in the quantity and quality of dietary protein intake.[13,14] On the one hand, exercise directly stimulates post-exercise muscle protein synthesis.[13] On the other hand, recent evidence shows that the current official nutritional recommendations for protein intake in older adults are insufficient to cause appreciable protein synthesis that reduces the risk of sarcopenia.[15–19] These recommendations range from 0.8 to 1.2 g/kg of body weight per day equally for all age groups, regardless of sex, physical activity or health status. However, it has been found that a daily protein intake of at least 1.2–1.5 g/kg of weight distributed throughout the day is safe and beneficial in the older adult population.[14,16] As a result, intake of around 20–30 g of protein of high biological value (10–15 g of essential amino acids with at least 3 g of leucine), preferably of animal origin, distributed across the three main meals (breakfast, lunch and dinner) has been suggested as one of the main effective strategies for counteracting protein catabolism and age-related muscle atrophy.[20,21]

Intake of proteins, in particular supplementation with leucine, an essential branched-chain amino acid, is among the most commonly used strategies to increase muscle protein synthesis, since it causes a more significant increase in expression of the nutrient-regulated signalling pathway called mammalian target of rapamycin complex 1 (mTORC1).[21,22] The mTOR signalling pathway is involved in various cell functions such as protein synthesis and autophagy.[21,22] Similarly, intake of leucine-rich foods causes a change in replacement of proteins in skeletal muscle and a decrease in proteolysis through stimulation of the signalling axis formed by peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α), which, among other functions, is responsible for regulating mitochondrial biogenesis, protein degradation and autophagy, as well as the signalling axis related to adenosine monophosphate-activated protein kinase (AMPKα) and sirtuin 1 (SIRT1) (SIRT1–AMPKα), both forming the SIRT1–AMPKα–PGC-1α axis.[21,22]

Like leucine (an amino acid), β-hydroxy-β-methylbutyrate (HMB), an active metabolite deriving from leucine catabolism, is involved as an adjuvant substance in preventing muscle atrophy due to disuse, as well as improving skeletal muscle recovery, a particular characteristic of older adults with sarcopenia. The primary function of HMB is to increase protein synthesis by activating the mTOR signalling pathway and insulin-like growth factor-1 (IGF-1), as well as anti-catabolic functions by decreasing the ubiquitin–proteasome pathway and increasing protein anabolism.[21,22]

Therefore, administration of foods along with leucine supplements or leucine-enriched protein supplements, and exercise, represent promising therapeutic measures in the treatment of factors leading to sarcopenia, such as muscle strength, muscle mass and physical fitness,[23] since various studies have found that leucine administration alone does not yield the same outcomes as leucine administration combined with protein supplements and exercise.[22,23]

Hence, this systematic review was conducted with the objective of identifying and evaluating the effects of supplementation with leucine, alone or combined with another supplement and with or without exercise in older adults with sarcopenia. The secondary objectives were to identify the exact amounts of leucine in the different types of intervention and to determine the type of exercise that synergistically causes improvements in the main markers of sarcopenia.

## Materials and methods

The protocol for this systematic review was recorded in the PROSPERO database in May 2021 (CRD42021243674). This systematic review was designed and developed in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) criteria for performing systematic reviews and meta-analyses.[24] These are a set of minimum evidence-based elements for evaluating the harms and benefits of a potential health intervention.

In order to find a specific response in the field of nutrition and clinical practice, and the best information search and analysis, several scientific databases were reviewed. To access the databases, the following libraries were used: PubMed/MEDLINE, the Cochrane database and SciELO for all entries up to 30 December 2020. English and Spanish were the two languages preferred in selecting studies. In order to increase the power of the analysis, no filters were applied with regard to race or sex.

The main terms used for the search strategy in the three databases were *sarcopenia, leucine* and *elderly (sarcopenia* [MeSH Terms] OR *sarcopenia* [All Fields]) AND *Sarcopenia* [Mesh] AND (*leucine* [MeSH Terms] OR *leucine* [All Fields]) AND (*aged* [MeSH Terms] OR *aged* [All Fields] OR *elderly* [All Fields]), as well as one of the following terms on a secondary basis: *older, trial, review* or *animals*.

To select the studies included in this systematic review, the titles and abstracts of each of the entries retrieved in the search were reviewed, with full examination of the studies in which the participants thoroughly met all the inclusion

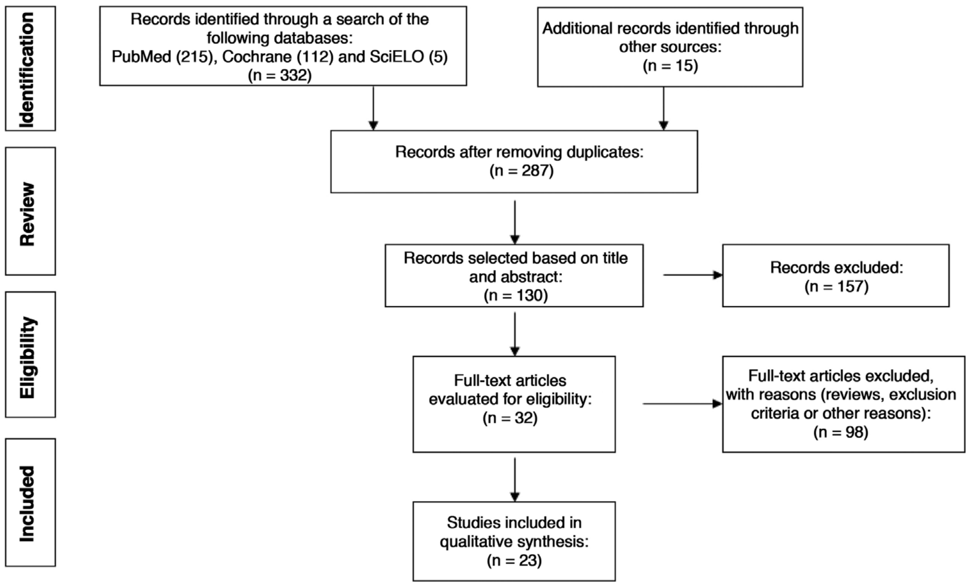


**Figure 1** Flow chart showing the results of the search for and retrieval of studies for the systematic review based on the inclusion and exclusion criteria.

criteria: a) experimental studies, randomised controlled trials and observational studies such as cohort, cross-sectional and case–control studies in older adults with sarcopenia; b) studies measuring at least one of the diagnostic criteria for sarcopenia, such as muscle mass and/or muscle strength and/or physical fitness; c) experimental studies on the effects of oral supplementation with leucine alone without exercise, oral supplementation with leucine plus exercise, oral supplementation with leucine plus another nutrient without exercise and oral supplementation with leucine plus other nutrients and exercise in the treatment of older adults with sarcopenia; and d) experimental studies in which the leucine dose administered to the subjects was specified or could be calculated.

As with the inclusion criteria, the following exclusion criteria were used to eliminate articles that did not meet the requirements: a) studies in which the study population was under 60 years of age; b) studies that did not include a placebo group; c) studies with a duration of less than a week; and d) studies conducted in animals with results used in humans to treat older adults with sarcopenia.

To determine and select studies aligned with the primary objective of this review for inclusion therein, each title/abstract was selected and reviewed independently, with full-text retrieval based on the inclusion and exclusion criteria specified for independent, individual review. Cross-references were made jointly to identify and exclude duplicate articles.

The methodological quality of the trials was assessed using the Cochrane Collaboration's tool for assessing risk of bias in randomised trials from the Cochrane Handbook, Version 5.10,[25] through evaluation of bias. A low risk of bias was assigned to studies that appropriately addressed each domain presented in the table of domains of bias, and a high risk of bias was assigned to studies that did not. An ''unclear risk of bias'' was assigned to studies in which not enough information was available to assess of risk of bias. The evaluation included the following domains of bias: random sequence generation, allocation concealment, blinding of participants, staff and outcome assessors, missing outcome data, selective reporting of results and other sources of bias.

## Results

The search strategy used initially identified a total of 332 articles from the three databases used: PubMed (215 articles), Cochrane (112 articles) and SciELO (five articles). In addition, the literature was manually searched for 15 relevant trials; this did not yield any additional studies for inclusion. After applying the inclusion and exclusion criteria, eliminating repeated articles, and analysing and reading the selected articles, a total of 23 studies were included in this systematic review. Fig. 1 shows the flow chart that lays out the strategy for searching for, selecting and eliminating articles and including articles meeting the above-mentioned eligibility criteria.

The 23 studies included were published between 2008 and 2020 (Tables 1 and 2). All older adults had been diagnosed with sarcopenia and were over 60 years of age.

Table 1 and Table 2 summarise the main characteristics of the 23 studies selected. The information shown in the two tables refers to: study source (author[s] and year of publication); study type; age, sex and number of participants in the study population; intervention type (dose of leucine and of other ingredients, protocol for administration, performance of exercise and duration and type of exercise), and outcomes achieved following oral supplementation with leucine alone or with exercise (Table 1) or oral supplementation with leucine plus another nutrient or plus another nutrient and exercise (Table 2).

The main parameters analysed to identify improvements in sarcopenia in the interventions made were muscle mass and muscle strength, since both are key components

**Table 1** Studies including leucine supplementation alone with exercise and leucine supplementation without exercise.

| Study, year (ref.) | Sample size (n) men/ women | Age (years) | Amount of leucine | Supplementation time | Exercise yes/no | Duration | Type of exercise | Outcome |
|---|---|---|---|---|---|---|---|---|
| Verhoeven et al. 2009/RCT[26] | n: 30<br>M: 30/W: 0 | ≥70 | 7.5 g/day | 12 weeks | NO | – | – | ↔ MM<br>↔ MS<br>↔ IS |
| Leenders et al. 2011/RCT[27] | n: 60<br>M: 60/W: 0 | ≥70 | 7.5 g/day | 24 weeks | NO | – | – | ↔ MM<br>↔ MS<br>↔ BF |
| Martínez et al. 2020/RCT[28] | n: 42<br>M: 14/W: 29 | ≥65 | 6 g/day | 6 weeks | NO | – | – | ↑ MM<br>↑ FU |
| Jacob et al. 2019/RCT[29] | n: 19<br>M: 0/W: 19 | ≥75 | 7.5 g/day | 12 weeks | YES | 60 min. Daily | 5 min WU + 3 sers × 15 reps (LPs, L-EXTs, BPs, CPs) +5 min STR | ↑ MM<br>↑ FU<br>↓ BF |

BF: body fat; BMD: bone mineral density; BPs: bench presses; Ca: calcium; CHs: carbohydrates; CPs: chest pulls; EAAs: essential amino acids; ETEs: endurance-training exercises; F: fat; FU: functionality; IS: insulin sensitivity; LCTs: long-chain triglycerides; L-EXTs: leg extensions; LPs: leg presses; M: men; MCTs: medium-chain triglycerides; MM: muscle mass; MS: muscle strength; n: sample size; RCT: randomised controlled trial; reps: repetitions; sers: series; STEs: strength-training exercises; STR: stretching; W: women; WU: warm-up; vit. D: vitamin D; ↑: increase; ↓: decrease; ↔: no change.

in determining sarcopenia. Other parameters related to improvements in quality of life in older adults with sarcopenia and a prognosis of improvements in health were also taken into account: bone mineral density, body fat, functionality, insulin sensitivity, endurance, aerobic capacity, inspiratory muscle strength and duration of rehabilitation (Tables 1 and 2).

In studies in which, along with supplementation, whether with leucine alone or leucine plus another nutrient, participants engaged in exercise regardless of type or duration, or did not engage in exercise, nutritional supplementation was maintained for the duration of the intervention (Tables 1 and 2).

Of the 23 studies included, those conducted by Verhoeven et al. and Leenders et al. which offered leucine supplements alone, without exercise, showed no improvements in muscle mass or strength.[26,27] Only a study by Martínez-Arnau et al.[28] found that the group who received supplementation with leucine alone, without exercise, did show small but significant effects on muscle mass index, since this was maintained for the duration of the intervention whereas the placebo group showed a decrease in muscle mass index. This effect of keeping muscle mass stable over time was related to improvements in nutritional status in individuals supplemented with leucine at the end of the intervention. On the other hand, a significant improvement in physical fitness, measured in terms of walking time, was seen after leucine supplementation compared to baseline in the intervention group; no effects were seen in the control group supplemented with placebo. By contrast, combining leucine with strength training showed significant improvements in markers of sarcopenia such as muscle strength and muscle mass, secondary to a decrease in body fat with no changes in insulin sensitivity levels.[29]

Of the seven studies that included oral supplementation of leucine plus another nutrient, without exercise,[30–36] four showed improvements in muscle mass and strength with a combination of leucine and other supplements such as essential amino acids, milk serum protein, carbohydrates, carnitine, creatine and medium-chain triglycerides.[32,34–36] A study conducted by Solerte et al.,[30] which studied only the effects of supplementation with leucine-enriched essential amino acids without exercise, found improvements in muscle mass, as well as a decrease in body fat and an improvement in insulin sensitivity as secondary improvements.[30] A study by Abe et al.[33] detected improvements in muscle strength, as well as in functionality on a secondary basis, following supplementation with leucine, vitamin D and medium-chain triglycerides.[33] The studies that included 400–800 IUs of vitamin D per day in addition

**Table 2** Studies including supplementation with leucine plus another nutrient and no exercise and supplementation with leucine plus other nutrients and exercise.

| Study, year (ref.) | Sample size (n) men/women | Age (years) | Supplement used (g/day) | Supplementation time | Exercise yes/no | Duration | Type of exercise | Outcome |
|---|---|---|---|---|---|---|---|---|
| Solerte et al. 2008/RCT[30] | n: 41<br>M: 41/W: 0 | 66–84 | 8 g EAAs (2.5 g LEU) | 18 months | NO | – | – | ↑ MM<br>↓ BF<br>↑ IS |
| Ferrando et al. 2009/RCT[31] | n: 22<br>M: 7/W: 15 | ≥65 | 3 × 15 g EAAs (5.3 g LEU) | 10 days | NO | – | – | ↔ MM<br>↔ MS |
| Bauer et al. 2015/RCT[32] | n: 380<br>M: 131/W: 249 | ≥65 | 2 × (20 g S-Prot + 3 g LEU + 9 g CHs + 800 IU vit. D + vitamins and minerals) | 13 weeks | NO | – | – | ↑ MS<br>↑ MM<br>↑ FU |
| Abe et al. 2016/RCT[33] | n: 38<br>M: 11/W: 27 | ≥85 | 1.2 g LEU + 800 IU vit. D + 6 g MCTs | 12 weeks | NO | – | – | ↑ MS<br>↑ FU |
| Evans et al. 2017/RCT[34] | n: 42<br>M: 15/W: 27 | 55–70 | 2.2 g CAR + 3 g CREAT + 2 g LEU + 400 IU vit. D | 8 weeks | NO | – | – | ↑ MS<br>↑ MM |
| Chanet et al. 2017/RCT[35] | n: 24<br>M: 0/W: 24 | ≥70 | 2 × (21 g S-Prot + 3 g LEU + 9 g CHs + 3 g F + 800 IU vit. D + vitamins and minerals) | 6 weeks | NO | – | – | ↑ MS<br>↑ FU<br>↑ MM |

Table 2 (*Continued*)

| Study, year (ref.) | Sample size (n) men/women | Age (years) | Supplement used (g/day) | Supplementation time | Exercise yes/no | Duration | Type of exercise | Outcome |
|---|---|---|---|---|---|---|---|---|
| Hill et al. 2019/RCT[36] | n: 380<br>M: 131/W: 249 | ≥65 | 2 × (20 g S-Prot + 3 g LEU + 9 g CHs + 800 IU vit. D +500 mg Ca + vitamins and minerals) | 13 weeks | NO | – | – | ↑ MS<br>↑ MM<br>↑ FU<br>↑ BMD |
| Kim et al. 2012/RCT[37] | n: 155<br>M: 0/W: 155 | ≥75 | 6 g EAAs (42% LEU [2.5 g]) | 12 weeks | YES | 60 min. Times/week not specified | 5 min WU +30 min EX (elast. band: hip/bi/tri-flex, K-EXTs) | ↑ MS<br>↑ MM<br>↑ FU |
| Rondanelli et al. 2016/RCT[21] | n: 130<br>M: 53/W: 77 | ≥80 | 22 g S-Prot + 10.9 g EAAs (4 g LEU), 4.4 g CHs + 100 IU vit. D | 12 weeks | YES | 20 min, 5 times/week | 5 min WU +5 min STEs (8 reps per leg, K-EXTs, L-EXTs, hip/bi/tri-flex) +5 min AEx +5 min STR | ↑ MS<br>↑ MM<br>↑ FU |
| van de Bool et al. 2017/RCT[38] | n: 81<br>M: 41/W: 40 | ≥60 | 3 × (4.2 g S-Prot + 4.2 g CAS + 0.09 g/kg/day LEU + 28.3 g CHs + 4.2 g F + vitamins and minerals | 16 weeks | YES | Not specified | Strength training exercises and aerobics. Not specified | ↑ MS<br>↑ AE<br>↑ IMS |
| Amasene et al. 2019/RCT[39] | n: 28<br>M: 0/W: 28 | ≥70 | 20 g S-Prot (3 g LEU) | 12 weeks | YES | 60 min<br>2 times/week | EX (hip/bi/tri-flex, K-EXTs, K-EXTs) + BEs +5 min STR Personalised load | ↑ MS<br>↑ MM |

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Table 2 (*Continued*)

| Study, year (ref.) | Sample size (n) men/women | Age (years) | Supplement used (g/day) | Supplementation time | Exercise yes/no | Duration | Type of exercise | Outcome |
|---|---|---|---|---|---|---|---|---|
| Kirk et al. 2019/RCT[40] | n: 46<br>M: 25/W: 21 | ≥65 | 1.5 g/kg/day S-Prot + 0.09 g/kg/day LEU | 16 weeks | YES | 2 times/week STEs + 1 time/week FEs | 8 STEs (BPs, LPs, HPs, SQs, CPs, bi/tri-flex) + 12 FEs | ↔ MM<br>↔ MS<br>↑ AC |
| Randolph et al. 2019/RCT[41] | n: 42<br>M: 13/W: 29 | 65–85 | 12.5 g EAAs (6 g LEU) | 22 weeks | YES | 45 min<br>3 times/week | Treadmill | ↑ MS<br>↑ AE<br>↑ FU |
| Yoshimura Y. et al. 2019/RCT[42] | n: 44<br>M: 14/W: 30 | ≥75 | 7 g EAAs (3 g LEU) + 9.7 g CHs. | 8 weeks | YES | Not deter-mined. Daily | Standing, handrail (2 series × 10 reps up to 120 reps). | ↑ MS<br>↑ MM<br>↑ AE<br>↑ FU |
| Moriwaki et al. 2019/RCT[43] | n: 55<br>M: 30/W: 15 | <75 and ≥75* | 2 × (2.5 g BCAAs [1.4 g LEU] + 400 IU vit. D) | 12 weeks | YES | 90 min | Standing, walking, rehabilitation room (exercise not specified) | ↑ MM<br>↑ BW |
| Kirk et al. 2020/RCT[44] | n: 100<br>M: 48/W: 52 | ≥65 | 1.5 g/kg/day S-Prot + 0.09 g/kg/day LEU | 16 weeks | YES | 2 times/week STEs + 1 time/week FEs | 8 STEs (BPs, LPs, HPs, SQs, CPs, bi/tri-flex) + 12 FEs | ↔ MM<br>↔ MS<br>↑ AC |

Table 2 (*Continued*)

| Study, year (ref.) | Sample size (n) men/women | Age (years) | Supplement used (g/day) | Supplementation time | Exercise yes/no | Duration | Type of exercise | Outcome |
|---|---|---|---|---|---|---|---|---|
| Yamamoto et al. 2020/RCT[45] | n: 53 | 70–79 | 2 × 3 g Prot (1.2 g LEU + 1.8 g OAAs + 0.04 g F + 0.2 g CHs) | 48 weeks | YES | 15 min/day | Elast. band 1 × 20 reps (COs + FARs + BCs + L-EXTs + CRs + SQs) | ↑ MM |
| | M: 28/W: 25 | | | | | 7 times/week STEs | | ↑ MS |
| Kang et al. 2020/RCT[46] | n: 120 | ≥65 | 2 × 20 g Prot-M (50% CAS + 40% Prot-S + 10% S (3 g LEU total) + 800 IU vit. D + 300 g Ca + 1.1 g F + 2.5 g CHs) | 12 weeks | YES | 20 min/day | Exercises not specified | ↑ MM |
| | M: 23/W: 87 | | | | | 7 times/week STEs | | ↑ MS |
| Rondanelli et al. 2020/RCT[47] | n: 140 | ≥65 | 2 × 40 g Pow-M (20 g S-Prot + 2.8 g LEU + 9 g CHs + 800 IU vit. D +500 mg Ca + FIB | 8 weeks | YES | 20–30 min/day | 5 min WU + 5–10 min STEs (8 reps: TRs; CRs; K-EXTs; CRs; se-K-flex/ex; st-K-flex/ex; L-EXTs; hip-flex; bi-flex) + 5–10 min BEs +5 min STR | ↑ MM |
| | M: 140/W: 0 | | | | | 5 times/week 20–30 min STEs | | ↑ MS |
| | | | | | | | | ↑ AE<br>↓ DRE |

AC: aerobic capacity; AE: aerobic endurance; AEx: aerobic exercise; BEs: balance exercises; BCs: biceps curls; BCAAs: branched-chain amino acids; BF: body fat; bi-flex: biceps flexion; bi/tri-flex: biceps/triceps flexion; BMD: bone mineral density; BPs: bench presses; BW: body weight; Ca: calcium; CAR: carnitine; CAS: casein; CHs: carbohydrates; COs: chest openers; CPs: chest pulls; CRs: calf raises; CREAT: creatine; DRE: duration of rehabilitation; EAAs: essential amino acids; elast. band: elastic band; ETEs: endurance training exercises; EX: exercise; F: fat; FARs: front arm raises; FEs: functional exercises; FIB: fibre; FU: functionality; g: gram; hip/bi/tri-flex: hip/biceps/triceps flexion; hip-flex: hip flexion; HPs: horizontal presses; IMS: inspiratory muscle strength; IS: insulin sensitivity; IU: international units; K-EXTs: knee extensions; KRs: knee raises; LEU: leucine; L-EXTs: leg extensions; LCTs: long-chain triglycerides; LLRs: lateral leg raises; LPs: leg presses; M: men; MCT: medium-chain triglycerides; mg: milligrams; MM: muscle mass; MS: muscle strength; MSM: muscle strength maintenance; n: sample size; OAAs: other amino acids; Pow-M: powder mixture; Prot-M: protein mixture; RCT: randomised controlled trial; reps: repetitions; S: soya; se-K-flex/ex: seated knee flexion/extension; sers: series; S-Prot: serum protein; SQs: squats; STEs: strength-training exercises; st-K-flex/ex: standing knee flexion/extension; STR: stretching; TRs: toe raises; W: women; WU: warm-up; vit. D: vitamin D; ↑: increase; ↓: decrease; ↔: no change/maintenance.

* Subjects were stratified by age into two subgroups.

to leucine-enriched milk serum protein showed not only an increase in muscle mass and strength, but also improvements in bone mineral density[32] and functionality,[32,33,35,36] thus achieving a better quality of life. Just a study by Ferrando et al.[31] offering a supplement that consisted of leucine-enriched essential amino acids, and no exercise, found that participants showed no improvements in muscle mass or strength when the data collected at the end of the study were compared to those collected at the start of the study.[31]

Studies that included supplements with leucine plus another nutrient and exercise showed significant improvements in muscle mass, muscle strength and functionality as well as a reduction in duration of rehabilitation at hospitals for older adults, especially those that combined supplementation and strength training.[21,37–48] A study by Rondanelli et al.[47] was the only one that, along with an increase in muscle mass and strength as well as aerobic endurance, found improvements in terms of a reduced duration of rehabilitation following supplementation with a mixture of proteins enriched with leucine, carbohydrates, vitamin D, calcium and dietary fibre. In a study conducted by van de Bool et al.,[38] participants showed not only improvements in muscle strength, but also other added functional improvements, such as improvements in inspiratory muscle strength and aerobic endurance, following addition of casein and fats along with leucine-enriched milk serum protein plus exercise. In just two studies, both conducted by Kirk et al. in different years,[41,43] muscle strength and mass outcomes remained the same at the start and the end of the intervention; only aerobic capacity improved.

Fig. 2 shows the analysis of the methodological quality and the risk of bias of the studies included.

| Study | Random sequence generation | Allocation concealment | Blinding of participants and staff | Blinding of outcome assessors | Missing outcome data | Selective reporting of results | Other sources of bias |
|---|---|---|---|---|---|---|---|
| Verhoeven *et al.* 2009 | – | ? | – | – | ? | – | – |
| Leenders *et al.* 2011 | + | + | – | ? | ? | – | – |
| Martínez y col. 2020 | – | – | ? | – | – | – | – |
| Jacob *et al.* 2019 | – | – | – | – | – | – | – |
| Solerte *et al.* 2008 | – | ? | ? | ? | ? | – | – |
| Ferrando *et al.* 2009 | – | – | – | ? | – | – | – |
| Bauer *et al.* 2015 | – | – | – | ? | – | – | – |
| Abe *et al.* 2016 | – | + | ? | ? | – | ? | – |
| Evans *et al.* 2017 | ? | – | – | ? | – | ? | – |
| Chanet *et al.* 2017 | – | – | – | + | + | – | – |
| Hill *et al.* 2019 | – | – | – | – | – | – | – |
| Kim *et al.* 2012 | – | – | – | ? | – | ? | – |
| Rondanelli *et al.* 2016 | – | – | – | ? | ? | – | – |
| Van de Bool *et al.* 2017 | – | – | – | ? | – | – | – |
| Amasene *et al.* 2019 | – | ? | ? | ? | ? | – | – |
| Kirk *et al.* 2019 | – | – | ? | ? | ? | – | – |
| Randolph *et al.* 2019 | – | ? | ? | ? | – | – | – |
| Yoshimura *et al.* 2019 | – | – | – | – | – | – | – |
| Moriwaki *et al.* 2019 | – | ? | ? | – | – | – | – |
| Kirk *et al.* 2020 | – | – | – | – | ? | – | – |
| Yamamoto *et al.* 2020 | – | ? | – | – | ? | ? | – |
| Kang *et al.* 2020 | – | – | – | – | ? | – | – |
| Rondanelli *et al.* 2020 | – | – | – | – | – | – | – |

| – | ? | + |
|---|---|---|
| Low risk | Unclear risk | High risk |

**Figure 2** Summary of risk of bias: review of the opinion of the different authors on each element of risk of bias for each of the studies included. The minus sign (−) indicates a low risk of bias, the plus sign (+) indicates a high risk of bias and the question mark (?) indicates an unclear risk of bias.

## Discussion

Loss of muscle mass and strength, as well as the functional consequences thereof particular to sarcopenia, are seen to a greater or lesser degree as an integral part of the normal changes that occur in the process of normal ageing,[2,3] as well as in certain diseases that increase the duration of bed rest.[23] One of the main problems seen in sedentary older adults is that they have certain limitations or lack initiative when it comes to exercising; in this case, nutritional intervention, in particular leucine supplementation, is one of the main actions taken to prevent the onset of sarcopenia, as well as the consequences thereof.[23,28] However, the results of this review indicate that administration of leucine in isolation does not yield the same outcomes as administration of leucine plus other supplements or plus exercise. Hence the analysis of the different studies reviewed found that nutritional interventions featuring leucine alone without other nutrients and without exercise did not improve sarcopenia-related issues.[26,27]

Just one study conducted by Martínez-Arnau et al.[28] found that participants who consumed 6 g of leucine/day maintained their lean muscle mass for more prolonged periods of time, such that nutritional status improved in individuals who received supplementation despite not engaging in exercise of any kind. This could be a good strategy in older adults with sarcopenia who are immobilised for different reasons, especially hospitalised older adults. By contrast, studies that did include exercise plus supplements with leucine, alone or together with another supplement, showed improvements in both muscle mass and strength, functionality, insulin sensitivity, body composition (reduction in body fat) and bone mineral density, in particular those whose formulas included creatine, carnitine and vitamin D as well as leucine.[26,29,32–36]

Studies that included supplements with leucine plus another supplement, as well as exercise, especially strength training, were those that showed significant increases and improvements in amounts of muscle mass and strength, translating to reduced comorbidities, falls and fractures and therefore a better quality of life for participants.[21,37–39,41–43,45–48] It should be noted that the studies that provided participants with supplementation with nutritional formulas based on milk serum protein enriched with leucine and vitamin D, with or without exercise, were the ones that showed the best outcomes, in terms of both primary sarcopenia markers, such as muscle mass, muscle strength and physical fitness, and secondary markers, such as bone mineral density, functionality, body weight, and inspiratory muscle strength.[21,32–36,43,46,47] Therefore, reversal of vitamin D deficiencies through supplementation of vitamin D combined with leucine and milk serum protein could contribute to favourable effects on the primary muscle parameters of sarcopenia, as well as the secondary markers. Similarly, hospitalised older adults who received nutritional supplements of milk serum protein enriched with leucine and vitamin D, or supplements of branched-chain amino acids enriched with leucine and vitamin D, showed not only an increase in muscle mass and strength, but also improvements in physical fitness and performance status, resulting in a reduction in duration of rehabilitation and a higher proportion of hospital discharges.[21,39,43] Therefore, it has been suggested that a nutritional supplement consisting of milk serum protein enriched with leucine and vitamin D nearly doubles postprandial muscle protein synthesis in an acute setting, thus improving markers of sarcopenia and attenuating inflammation, which is the key factor contributing to the disease.[21,39,43]

Like supplementation with whey enriched with leucine and vitamin D, supplementation with creatine plus L-carnitine and leucine also showed significant improvements in total muscle mass as well as muscle strength.[34]. As demonstrated by Evans et al.,[34] adding leucine and creatine can have synergistic effects when combined with L-carnitine, yielding improvements in the primary markers of sarcopenia, regardless of whether exercise is done. This can likely be explained by a common mechanism that promotes increased protein synthesis, increases branched-chain amino acid bioavailability and decreases protein degradation.[34]

Neither the duration nor the volume of series in each training session were determinant of improvements in the main markers of sarcopenia in the non-institutionalised older adults included in the various interventions reviewed.[21,37–39,41–43,45–48] By contrast, hospitalised older adults who were administered oral supplementation of leucine plus another nutrient showed significant improvements in functional capacity (including gait and walking ability) and muscle mass and strength as both the intensity and the duration of the session increased in the training sessions; this resulted in a decrease in rehabilitation time, an increase in earlier hospital discharges and a decrease in healthcare costs.[42,43,47]

Regarding the duration of the intervention, studies such as those by Luiking et al.,[49] Holwerda et al.[50] and Churchward-Venne et al.[51] found that interventions lasting less than a week improved issues related to protein synthesis and muscle mass only; they did not improve muscle strength or quality of life. Therefore, taking into account the results of this review, it seems that the duration of treatment with leucine should be longer than a week for long-term effects in terms of improvements in the parameters of muscle mass, muscle strength and functionality to occur in older adults with sarcopenia, although more studies are needed to confirm these findings.

The studies presented in the review included adults over 60 years of age who were in hospitals, communities for elderly people and their own homes, which reaffirms the idea that the results present in this review could be applied to the majority of those who suffer from sarcopenia.

The use of nutritional strategies rich in supplements containing leucine as part of their formulas, along with exercise, in particular strength training, has been seen to significantly improve the markers of sarcopenia, as well as quality of life. This improvement has been independent of the duration and the volume of training sessions, especially in older adults, both outpatient and non-hospitalised. The exact dose of leucine that the supplements should contain has yet to be determined, since improvements have been observed in both studies offering 2.5 g/day of leucine as an integral part of their formulas and studies offering up to 6 g/day. In addition, treatments with leucine alone as nutritional strategies do not currently yield significant improvements in preventing or treating sarcopenia in older adults, since only modest maintenance of mass and muscle strength for the duration of the intervention has been observed.

The scientific literature features studies reporting how leucine supplementation alone ensures maintenance of muscle strength and mass for the duration of the intervention in older adults.[28] Some studies have also shown that, after exercise, accumulation of muscle proteins is stimulated in older adults, and that strength training is an effective treatment strategy to increase skeletal muscle mass and improve functional performance in this population.[13,14] However, no systematic review published to date has included studies separately comparing the influence of supplementation with leucine alone, supplementation with leucine plus another nutrient and supplementation with leucine plus exercise in the treatment of older adults with sarcopenia.

The studies included in this review featuring supplementation with leucine alone were very few in number; therefore, more evidence is needed to establish a dose of leucine alone as part of the treatment and prevention of sarcopenia. Hence, further research is needed in this area to determine an exact dose of supplementation with leucine alone and to assess the impact of leucine supplementation in preventing sarcopenia.

## Conclusions

Physical inactivity, together with inadequate intake of nutrients, especially proteins of high biological value, can lead to an increased risk of suffering from sarcopenia. A suitable diet featuring intake of high-quality, leucine-rich protein, plus exercise, in particular strength training, represents one of the most effective strategies to delay the onset of sarcopenia, as well as the events associated with this condition.

The use of supplementation containing protein, leucine and vitamin D, among other supplements, exerts effects of protection and maintenance on skeletal muscle, especially muscle mass and strength, thus substantially improving quality of life in older adults, regardless of whether or not they exercise. Similarly, in older adults with sarcopenia admitted to hospitals for rehabilitation, the use of supplementation based on milk serum protein enriched with leucine and vitamin D is proposed as a promising treatment to improve physical fitness, physical function and muscle mass, as well as reduce hospital costs, in older adults with sarcopenia.

Oral supplementation of leucine along with L-carnitine and creatine is proposed as another promising basis for supplementation in improving sarcopenia.

To date, administration of supplements with leucine alone, without exercise, as part of the treatment of sarcopenia has not shown enough significant improvements in markers of sarcopenia to include it in the nutritional strategy for the treatment of the disease.

The appropriate amount of leucine to be included in the nutritional supplements offered, whether alone, with exercise or enriched with milk serum protein or other nutrients, remains to be determined. However, the results of the different studies included in the review justify the conduct of further research on the role of specific nutritional supplements as part of treatment for the prevention of adverse outcomes in older adults at risk of sarcopenia.

Regarding strength training, a gradual increase in intensity as strength increases has been shown to improve and/or preserve both muscle strength and mass. While the appropriate volume of training and the type of exercise causing significant improvements in markers of sarcopenia are not clear, there is disagreement as to whether exercise should be concentric or eccentric; therefore, further research is needed to arrive at proper guidelines for each training session, whether inpatient or outpatient.

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## Conflicts of interest

The authors declare that they have no conflicts of interest.

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