

Leucine Supplementation and Sarcopenia

Subjects: Nutrition & Dietetics

Contributor: Omar Cauli

Treating sarcopenia remains a challenge, and nutritional interventions present promising approaches. We summarize the effects of leucine supplementation in treating older individuals with sarcopenia associated with aging or to specific disorders, and we focus on the effect of leucine supplementation on various sarcopenia criteria, e.g., muscular strength, lean mass, and physical performance.

Keywords: leucine ; sarcopenia ; muscular mass ; muscular strength ; elderly

1. Introduction

Sarcopenia is defined as a progressive loss of muscle mass, strength, and function ^{[1][2]}. From a physiological point view, sarcopenia starts in the fifth decade of life and at a population level, proceeds at a rate of $\sim 0.8\%$ annually. In fact, the decrease in skeletal muscle strength induced by sarcopenia, known as dynapenia, is even more precipitous, occurring at an annual rate of $\sim 2\text{--}3\%$ ^{[2][3]} and it is estimated that more than 20% of adults aged over 65 years, and over 50% of those aged at least 80 years are sarcopenic ^[4]. In some pathological conditions such as liver cirrhosis ^[5], chronic obstructive pulmonary disease ^{[6][7]}, and diabetes ^[8], sarcopenia can appear earlier and its progression occurs more quickly. Several studies report that the most sarcopenic individuals are at an increased risk of developing severe sarcopenia-related complications, such as an increased risk of falls ^{[9][10]}, frailty ^[11], disability ^[12], and type-II diabetes ^{[13][14]}, which all have a negative impact on their quality of life and premature mortality.

Within the framework of the Third National Health and Nutrition Examination Survey, Srikanthan and Karlamangla ^[12] have demonstrated that muscle mass is a predictor of longevity when taking into account the all-cause mortality in North American adults (aged over 55 or 65 years for men and women, respectively). One of the main ways in which sarcopenia contributes to the disease is that it alters muscular turnover and metabolism ^{[15][16]}. Moreover, older adults exhibit a decreased anabolic response to protein feeding ^{[17][18]}, which is a mechanism underpinning the loss of muscle mass in sarcopenic individuals. Compared to younger adults, those aged over 65 years required $\sim 70\%$ more protein per meal to maximally stimulate muscle protein synthesis ^[19]. Furthermore, at a global level, only 40% of older adults meet the recommended daily allowance for protein ($0.8 \text{ g}\cdot\text{kg}^{-1}\cdot\text{d}^{-1}$) and 10% of older women do not even meet the estimated average requirement of $0.66 \text{ g}\cdot\text{kg}^{-1}\cdot\text{d}^{-1}$ ^{[20][21]}.

One strategy to increase the muscle protein synthesis that has been investigated is the supplementation of diets with leucine, an essential branched-chain amino acid with important regulatory actions in muscles, which are at least partially mediated by the mammalian target of the rapamycin pathway ^[22]. Leucine modifies protein turnover in skeletal muscles, by decreasing proteolysis and by increasing protein synthesis. Physiological research reports have shown that leucine can enhance muscle protein-synthesis ^{[23][24][25][26]}. Furthermore, leucine can stimulate insulin release by pancreatic cells ^[27], showing that besides its beneficial effect in enhancing skeletal muscle glucose uptake, it is also an important anabolic signal in skeletal muscle.

Based on the above, administration of leucine-containing supplements is therefore a promising approach for treating sarcopenia. We took a systematic approach to analysing the current scientific evidence in this area, and to ascertaining whether the administration of leucine-containing supplements is effective in the treatment of sarcopenia. We also included interventions that used whey protein as a supplement, because these contain large amounts of leucine (approximately 13 g leucine/100 g protein ^[28]) and the consumption of whey protein appears to be the most effective at increasing muscle protein synthesis ^[29].

2. Current Insights on Leucine Supplementation and Sarcopenia

The loss of muscle mass and strength (sarcopenia) and its functional consequences such as reduced walking speed can occur to a varying degree both as a part of physiological changes during aging ^[30] or in some pathological conditions

increasing the amount of time in bed which in itself can result in catabolism and a decrease in muscle mass [31][32] or patients with chronic diseases such as liver cirrhosis [5][33], COPD [6][7], diabetes [34] and post-stroke patients [35]. Severe sarcopenia is associated with an extremely high rate of disability but not necessarily with body-weight loss, and obese sarcopenic patients appear to have even worse outcomes [36][37][38].

The best approaches to treat sarcopenia or delay its progression over time are currently based on physical exercise and nutritional supplementation, with resistance training being the most useful tool for effectively preventing [39][40] and treating sarcopenia [41][42][43]. However, many older people are sedentary and either cannot (social barriers and family support) or do not want to exercise. In these cases, nutritional interventions remain the most promising intervention to delay the appearance of the adverse consequences of sarcopenia such as falls, mobility loss, and a bed-to-sofa lifestyle. Supplementation of the branched-chain amino acid, leucine, or leucine-enriched protein (whey/casein protein) [28][29][44] is one of the most common interventions for treating sarcopenia in older individuals, and several RCTs have been published in the last five years.

Analysis of the trials included in this systematic review showed that these nutritional interventions can improve several aspects of sarcopenia, including muscle strength, lean mass content, and walking speed (although the latter has seldom been investigated) although these effects depend on sarcopenia criteria (ranging from improvement to no effect) and the patients' characteristics. Only one study [45] found a reduction in muscle strength after supplementation with whey protein and casein, although they did not make the reasons for this reduction clear. As possible causes, they cited an insufficient difference in protein intake between groups, a lack of a need for protein-supplementation because of already sufficient dietary protein intake, or the specific characteristics of the patients included in the sample who all had polymyalgia rheumatica. In overall terms, the results of the review suggest that the beneficial effects of leucine-supplementation are more consistent for the improvement in lean mass reported in 63% of the analysed studies, which is consistent with the physiological action of leucine in muscle metabolism.

Interestingly, the majority of interventions that found an improvement in lean mass after leucine supplementation also co-administered vitamin D as a part of the nutritional intervention. Vitamin D is increasingly recognised as playing an important role in normal muscle function, and low vitamin D status is associated with an increased risk of falls and proximal weakness [46] and these effects are more prominent in male individuals [47]. In view of these sex-dependent effects, the differential effects of leucine supplementation with or without vitamin D and the measurement of vitamin D as a biological marker of sarcopenia need further research.

In contrast, the sarcopenia criteria related to muscle strength and physical performance are not only mediated by an efficient and proper lean mass, but also by a central nervous system component which forms a neuro-muscular (motor) unit [3][48]. Improvements in neuromuscular activation precede increases in muscle mass in response to resistance training; neuromuscular activation has therefore been proposed as another measure of muscle quality [48]. The fact that in most studies there were no clear parallelism between a positive effect in lean mass improvement accompanied by a positive effect on muscle strength or physical performance confirms that the central nervous system component is necessary for restoring muscular strength in age- and diseases-related sarcopenia is less sensitive to leucine supplementation than lean mass improvement.

With regards to the effects of leucine supplementation in sarcopenia occurring in individuals with some kind of disease, an improvement in muscle strength was observed in COPD patients [49]. Muscle wasting is common in COPD, particularly among those with the emphysema-type, and associated with a high prevalence of osteoporosis, impaired exercise performance, and higher mortality risk [50]. Further RCT should address the effects of leucine supplementation on physical performance, which may definitely be relevant to patients' ability to carry out basic activities of daily life and their quality of life.

A beneficial effect of leucine supplementation co-administered with a program of physical exercise was observed in post-stroke patients for both muscular strength and lean mass [35].

Regarding methodological issues, our analysis highlighted that different sarcopenia evaluation methods have often failed to adequately meet the criteria proposed by the international guidelines [1][51][52]. Therefore, future studies should focus on evaluating treatment effects using accepted criteria for defining sarcopenia and to select optimally the features of individuals or patients that could benefit more following an intervention consisting in leucine supplementation [51].

To the best of our knowledge, no published work has investigated the role of comorbidities or polymedication in the effects produced by leucine supplementation and as such further research in this area is clearly warranted. This would enable the selection of patients who could most benefit from supplementation, such as patients with COPD or cirrhosis, because

these diseases are classically associated with sarcopenia ^{[49][53][54]}. To date, the best available evidence suggests that administration of leucine-enriched proteins such as whey/casein protein or an EAA mixture, has beneficial effects for sarcopenic individuals, is well-tolerated, and does not have any serious adverse effects.

3. Conclusion

Finally, whenever possible, a multidisciplinary approach to treating sarcopenia must be encouraged. This means that besides leucine supplementation, clinicians should prescribe a balanced diet and physical exercise to treat this condition. Finally, our analysis also provides evidence that leucine or leucine-enriched protein supplementation may also have other beneficial effects, such as improving cognitive function ^{[49][55][53]}, symptoms of depression ^{[54][56]}, and quality of life ^{[57][49]}, which lends further support to the use of this intervention in geriatric populations.

References

1. Cruz-Jentoft, A.J.; Baeyens, J.P.; Bauer, J.M.; Boirie, Y.; Cederholm, T.; Landi, F.; Martin, F.C.; Michel, J.-P.; Rolland, Y.; Schneider, S.M.; et al. Sarcopenia: European consensus on definition and diagnosis: Report of the European Working Group on Sarcopenia in Older People. *Age Ageing* 2010, 39, 412–423.
2. Janssen, I. The epidemiology of sarcopenia. *Clin. Geriatr. Med.* 2011, 27, 355–363.
3. Tieland, M.; Trouwborst, I.; Clark, B.C. Skeletal muscle performance and ageing. *J. Cachexia Sarcopenia Muscle* 2018, 9, 3–19.
4. von Haehling, S.; Morley, J.E.; Anker, S.D. An overview of sarcopenia: Facts and numbers on prevalence and clinical impact. *J. Cachexia Sarcopenia Muscle* 2010, 1, 129–133.
5. Kim, G.; Kang, S.H.; Kim, M.Y.; Baik, S.K. Prognostic value of sarcopenia in patients with liver cirrhosis: A systematic review and meta-analysis. *PLoS ONE* 2017, 12, e0186990.
6. Bone, A.E.; Hepgul, N.; Kon, S.; Maddocks, M. Sarcopenia and frailty in chronic respiratory disease. *Chronic Respir. Dis.* 2017, 14, 85–99.
7. Jones, S.E.; Maddocks, M.; Kon, S.S.C.; Canavan, J.L.; Nolan, C.M.; Clark, A.L.; Polkey, M.I.; Man, W.D.-C. Sarcopenia in COPD: Prevalence, clinical correlates and response to pulmonary rehabilitation. *Thorax* 2015, 70, 213–218.
8. Umegaki, H. Sarcopenia and frailty in older patients with diabetes mellitus. *Geriatr. Gerontol. Int.* 2016, 16, 293–299.
9. Follis, S.; Cook, A.; Bea, J.W.; Going, S.B.; Laddu, D.; Cauley, J.A.; Shadyab, A.H.; Stefanick, M.L.; Chen, Z. Association Between Sarcopenic Obesity and Falls in a Multiethnic Cohort of Postmenopausal Women. *J. Am. Geriatr. Soc.* 2018, 66, 2314–2320.
10. Sim, M.; Prince, R.L.; Scott, D.; Daly, R.M.; Duque, G.; Inderjeeth, C.A.; Zhu, K.; Woodman, R.J.; Hodgson, J.M.; Lewis, J.R. Utility of four sarcopenia criteria for the prediction of falls-related hospitalization in older Australian women. *Osteoporos. Int.* 2019, 30, 167–176.
11. Marzetti, E.; Hwang, A.-C.; Tosato, M.; Peng, L.-N.; Calvani, R.; Picca, A.; Chen, L.-K.; Landi, F. Age-related changes of skeletal muscle mass and strength among Italian and Taiwanese older people: Results from the Milan EXPO 2015 survey and the I-Lan Longitudinal Aging Study. *Exp. Gerontol.* 2018, 102, 76–80.
12. Srikanthan, P.; Karlamangla, A.S. Muscle mass index as a predictor of longevity in older adults. *Am. J. Med.* 2014, 127, 547–553.
13. Cuthbertson, D.J.; Bell, J.A.; Ng, S.Y.; Kemp, G.J.; Kivimaki, M.; Hamer, M. Dynapenic obesity and the risk of incident Type 2 diabetes: The English Longitudinal Study of Ageing. *Diabet. Med.* 2016, 33, 1052–1059.
14. Srikanthan, P.; Hevener, A.L.; Karlamangla, A.S. Sarcopenia exacerbates obesity-associated insulin resistance and dysglycemia: Findings from the National Health and Nutrition Examination Survey III. *PLoS ONE* 2010, 5, e10805.
15. Deer, R.R.; Dickinson, J.M.; Fisher, S.R.; Ju, H.; Volpi, E. Identifying effective and feasible interventions to accelerate functional recovery from hospitalization in older adults: A randomized controlled pilot trial. *Contemp. Clin. Trials* 2016, 49, 6–14.
16. Morais, J.A.; Jacob, K.W.; Chevalier, S. Effects of aging and insulin resistant states on protein anabolic responses in older adults. *Exp. Gerontol.* 2018, 108, 262–268.
17. Breen, L.; Phillips, S.M. Skeletal muscle protein metabolism in the elderly: Interventions to counteract the “anabolic resistance” of ageing. *Nutr. Metab. (Lond.)* 2011, 8, 68.

18. Paddon-Jones, D.; Campbell, W.W.; Jacques, P.F.; Kritchevsky, S.B.; Moore, L.L.; Rodriguez, N.R.; van Loon, L.J. Protein and healthy aging. *Am. J. Clin. Nutr.* 2015, 101, 1339S–1345S.
19. Murphy, C.H.; Oikawa, S.Y.; Phillips, S.M. Dietary Protein to Maintain Muscle Mass in Aging: A Case for Per-meal Protein Recommendations. *J. Frailty Aging* 2016, 5, 49–58.
20. Bauer, J.; Biolo, G.; Cederholm, T.; Cesari, M.; Cruz-Jentoft, A.J.; Morley, J.E.; Phillips, S.; Sieber, C.; Stehle, P.; Teta, D.; et al. Evidence-based recommendations for optimal dietary protein intake in older people: A position paper from the PROT-AGE Study Group. *J. Am. Med. Dir. Assoc.* 2013, 14, 542–559.
21. Volpi, E.; Campbell, W.W.; Dwyer, J.T.; Johnson, M.A.; Jensen, G.L.; Morley, J.E.; Wolfe, R.R. Is the optimal level of protein intake for older adults greater than the recommended dietary allowance? *J. Gerontol. A Biol. Sci. Med. Sci.* 2013, 68, 677–681.
22. De Bandt, J.-P. Leucine and Mammalian Target of Rapamycin-Dependent Activation of Muscle Protein Synthesis in Aging. *J. Nutr.* 2016, 146, 2616S–2624S.
23. Casperson, S.L.; Sheffield-Moore, M.; Hewlings, S.J.; Paddon-Jones, D. Leucine supplementation chronically improves muscle protein synthesis in older adults consuming the RDA for protein. *Clin. Nutr.* 2012, 31, 512–519.
24. Wall, B.T.; van Loon, L.J.C. Nutritional strategies to attenuate muscle disuse atrophy. *Nutr. Rev.* 2013, 71, 195–208.
25. Rieu, I.; Balage, M.; Sornet, C.; Giraudet, C.; Pujos, E.; Grizard, J.; Mosoni, L.; Dardevet, D. Leucine supplementation improves muscle protein synthesis in elderly men independently of hyperaminoacidaemia. *J. Physiol. (Lond.)* 2006, 575, 305–315.
26. Balage, M.; Dardevet, D. Long-term effects of leucine supplementation on body composition. *Curr. Opin. Clin. Nutr. Metab. Care* 2010, 13, 265–270.
27. van Loon, L.J.C.; Kruijschoop, M.; Menheere, P.P.C.A.; Wagenmakers, A.J.M.; Saris, W.H.M.; Keizer, H.A. Amino acid ingestion strongly enhances insulin secretion in patients with long-term type 2 diabetes. *Diabetes Care* 2003, 26, 625–630.
28. Hamarsland, H.; Nordengen, A.L.; Nyvik Aas, S.; Holte, K.; Garthe, I.; Paulsen, G.; Cotter, M.; Børsheim, E.; Benestad, H.B.; Raastad, T. Native whey protein with high levels of leucine results in similar post-exercise muscular anabolic responses as regular whey protein: A randomized controlled trial. *J. Int. Soc. Sports Nutr.* 2017, 14, 43.
29. Tang, J.E.; Moore, D.R.; Kujbida, G.W.; Tarnopolsky, M.A.; Phillips, S.M. Ingestion of whey hydrolysate, casein, or soy protein isolate: Effects on mixed muscle protein synthesis at rest and following resistance exercise in young men. *J. Appl. Physiol.* 2009, 107, 987–992.
30. Beaudart, C.; Zaaria, M.; Pasleau, F.; Reginster, J.-Y.; Bruyère, O. Health Outcomes of Sarcopenia: A Systematic Review and Meta-Analysis. *PLoS ONE* 2017, 12, e0169548.
31. Cerri, A.P.; Bellelli, G.; Mazzone, A.; Pittella, F.; Landi, F.; Zambon, A.; Annoni, G. Sarcopenia and malnutrition in acutely ill hospitalized elderly: Prevalence and outcomes. *Clin. Nutr.* 2015, 34, 745–751.
32. Fielding, R.A.; Vellas, B.; Evans, W.J.; Bhasin, S.; Morley, J.E.; Newman, A.B.; Abellan van Kan, G.; Andrieu, S.; Bauer, J.; Breuille, D.; et al. Sarcopenia: An undiagnosed condition in older adults. Current consensus definition: Prevalence, etiology, and consequences. International working group on sarcopenia. *J. Am. Med. Dir. Assoc.* 2011, 12, 249–256.
33. Sinclair, M.; Gow, P.J.; Grossmann, M.; Angus, P.W. Review article: Sarcopenia in cirrhosis—Aetiology, implications and potential therapeutic interventions. *Aliment. Pharmacol. Ther.* 2016, 43, 765–777.
34. Leenders, M.; Verdijk, L.B.; van der Hoeven, L.; van Kranenburg, J.; Hartgens, F.; Wodzig, W.K.W.H.; Saris, W.H.M.; van Loon, L.J.C. Prolonged leucine supplementation does not augment muscle mass or affect glycemic control in elderly type 2 diabetic men. *J. Nutr.* 2011, 141, 1070–1076.
35. Yoshimura, Y.; Bise, T.; Shimazu, S.; Tanoue, M.; Tomioka, Y.; Araki, M.; Nishino, T.; Kuzuhara, A.; Takatsuki, F. Effects of a leucine-enriched amino acid supplement on muscle mass, muscle strength, and physical function in post-stroke patients with sarcopenia: A randomized controlled trial. *Nutrition* 2019, 58, 1–6.
36. Choi, K.M. Sarcopenia and sarcopenic obesity. *Korean J. Intern. Med.* 2016, 31, 1054–1060.
37. Poggiogalle, E.; Migliaccio, S.; Lenzi, A.; Donini, L.M. Treatment of body composition changes in obese and overweight older adults: Insight into the phenotype of sarcopenic obesity. *Endocrine* 2014, 47, 699–716.
38. Morley, J.E. Frailty and Sarcopenia: The New Geriatric Giants. *Rev. Investig. Clin.* 2016, 68, 59–67.
39. Makanae, Y.; Fujita, S. Role of Exercise and Nutrition in the Prevention of Sarcopenia. *J. Nutr. Sci. Vitaminol.* 2015, 61, S125–S127.
40. Coelho-Júnior, H.J.; de Oliveira Gonçalves, I.; Sampaio, R.A.C.; Sewo Sampaio, P.Y.; Cadore, E.L.; Izquierdo, M.; Marzetti, E.; Uchida, M.C. Periodized and non-periodized resistance training programs on body composition and physical function in older adults. *J. Sports Exerc.* 2018, 20, 105–115.

41. Osuka, Y.; Kojima, N.; Wakaba, K.; Miyauchi, D.; Tanaka, K.; Kim, H. Effects of resistance training and/or beta-hydroxy-beta-methylbutyrate supplementation on muscle mass, muscle strength and physical performance in older women with reduced muscle mass: Protocol for a randomised, double-blind, placebo-controlled trial. *BMJ Open* 2019, 9, e025723.
42. Tsuzuku, S.; Kajioka, T.; Sakakibara, H.; Shimaoka, K. Slow movement resistance training using body weight improves muscle mass in the elderly: A randomized controlled trial. *Scand. J. Med. Sci. Sports* 2018, 28, 1339–1344.
43. Komar, B.; Schwingshackl, L.; Hoffmann, G. Effects of leucine-rich protein supplements on anthropometric parameter and muscle strength in the elderly: A systematic review and meta-analysis. *J. Nutr. Health Aging* 2015, 19, 437–446.
44. Antoniak, A.E.; Greig, C.A. The effect of combined resistance exercise training and vitamin D3 supplementation on musculoskeletal health and function in older adults: A systematic review and meta-analysis. *BMJ Open* 2017, 7, e014619.
45. Björkman, M.P.; Pilvi, T.K.; Kekkonen, R.A.; Korpela, R.; Tilvis, R.S. Similar effects of leucine rich and regular dairy products on muscle mass and functions of older polymyalgia rheumatica patients: A randomized crossover trial. *J. Nutr. Health Aging* 2011, 15, 462–467.
46. Spira, D.; Buchmann, N.; König, M.; Rosada, A.; Steinhagen-Thiessen, E.; Demuth, I.; Norman, K. Sex-specific differences in the association of vitamin D with low lean mass and frailty: Results from the Berlin Aging Study II. *Nutrition* 2019, 62, 1–6.
47. McGregor, R.A.; Cameron-Smith, D.; Poppitt, S.D. It is not just muscle mass: A review of muscle quality, composition and metabolism during ageing as determinants of muscle function and mobility in later life. *Longev. Healthspan* 2014, 3, 9.
48. Häkkinen, K.; Kraemer, W.J.; Kallinen, M.; Linnamo, V.; Pastinen, U.M.; Newton, R.U. Bilateral and unilateral neuromuscular function and muscle cross-sectional area in middle-aged and elderly men and women. *J. Gerontol. A Biol. Sci. Med. Sci.* 1996, 51, B21–B29.
49. Dal Negro, R.W.; Testa, A.; Aquilani, R.; Tognella, S.; Pasini, E.; Barbieri, A.; Boschi, F. Essential amino acid supplementation in patients with severe COPD: A step towards home rehabilitation. *Monaldi Arch. Chest Dis.* 2012, 77, 67–75.
50. Schols, A.M.; Ferreira, I.M.; Franssen, F.M.; Gosker, H.R.; Janssens, W.; Muscaritoli, M.; Pison, C.; Rutten-van Mölken, M.; Slinde, F.; Steiner, M.C.; et al. Nutritional assessment and therapy in COPD: A European Respiratory Society statement. *Eur. Respir. J.* 2014, 44, 1504–1520.
51. Cruz-Jentoft, A.J.; Bahat, G.; Bauer, J.; Boirie, Y.; Bruyère, O.; Cederholm, T.; Cooper, C.; Landi, F.; Rolland, Y.; Sayer, A.A.; et al. Sarcopenia: Revised European consensus on definition and diagnosis. *Age Ageing* 2019, 48, 16–31.
52. Studenski, S.A.; Peters, K.W.; Alley, D.E.; Cawthon, P.M.; McLean, R.R.; Harris, T.B.; Ferrucci, L.; Guralnik, J.M.; Fraga la, M.S.; Kenny, A.M.; et al. The FNIH sarcopenia project: Rationale, study description, conference recommendations, and final estimates. *J. Gerontol. A Biol. Sci. Med. Sci.* 2014, 69, 547–558.
53. Soriano, G.; Román, E.; Torrades, M.T.; Cárdenas, G.; Córdoba, J.; Blanco, M.J.N.; Nieto, J.C.; Vidal, S.; Villanueva, C.; Ambrós, H.B.; et al. Estudio del beneficio de un programa de ejercicio físico y suplementos de aminoácidos ramificados en la dieta dirigido a pacientes con cirrosis hepática. *Trauma* 2013, 24, 6–11.
54. van de Bool, C.; Rutten, E.P.A.; van Helvoort, A.; Franssen, F.M.E.; Wouters, E.F.M.; Schols, A.M.W.J. A randomized clinical trial investigating the efficacy of targeted nutrition as adjunct to exercise training in COPD. *J. Cachexia Sarcopenia Muscle* 2017, 8, 748–758.
55. Rondanelli, M.; Klersy, C.; Terracol, G.; Talluri, J.; Maugeri, R.; Guido, D.; Faliva, M.A.; Solerte, B.S.; Fioravanti, M.; Lukaski, H.; et al. Whey protein, amino acids, and vitamin D supplementation with physical activity increases fat-free mass and strength, functionality, and quality of life and decreases inflammation in sarcopenic elderly. *Am. J. Clin. Nutr.* 2016, 103, 830–840.
56. Verlaan, S.; Maier, A.B.; Bauer, J.M.; Bautmans, I.; Brandt, K.; Donini, L.M.; Maggio, M.; McMurdo, M.E.T.; Mets, T.; Seal, C.; et al. Sufficient levels of 25-hydroxyvitamin D and protein intake required to increase muscle mass in sarcopenic older adults—The PROVIDE study. *Clin. Nutr.* 2018, 37, 551–557.
57. Bauer, J.M.; Verlaan, S.; Bautmans, I.; Brandt, K.; Donini, L.M.; Maggio, M.; McMurdo, M.E.T.; Mets, T.; Seal, C.; Wijers, S.L.; et al. Effects of a vitamin D and leucine-enriched whey protein nutritional supplement on measures of sarcopenia in older adults, the PROVIDE study: A randomized, double-blind, placebo-controlled trial. *J. Am. Med. Dir. Assoc.* 2015, 16, 740–747.

