Minireview *Highlight article*

Dietary protein and exercise for preservation of lean mass and perspectives on type 2 diabetes prevention

Maysa Vieira de Sousa[1], Diana Bento da Silva Soares[1], Elaine Reis Caraça[2] and Ronaldo Cardoso[1]

[1]Endocrinology Division, School of Medicine, University of São Paulo, São Paulo 01246-903, Brazil; [2]Organização Social de Saúde, Santa Marcelina de Itaquaquecetuba, SP 08599-280, Brazil

Corresponding author: Maysa Vieira de Sousa. Email: maysavsousa@gmail.com

Impact statement

Diabetes mellitus is a worldwide health problem associated with obesity and sedentary lifestyle, which predisposes affected individuals to mortality and morbidity. Additionally, aging and unhealthy lifestyle behaviors increase inflammation and insulin resistance, contributing to the reduction of cytokines related to muscle nutrition and the suppression of lipogenesis, resulting in the development of sarcopenic obesity. One strategy for the prevention of T2D is the avoidance of secondary aging by participating in healthy action programs, including exercise and nutritional interventions. This minireview of several studies demonstrates the impact of physical activity and nutritional interventions on gaining or preserving muscle mass and on the functional aspects of muscles with aging. It provides information on the effect of protein, leucine, β-hydroxy-β-methylbutyrate (HMB), and creatine supplementation on muscle mass, strength, and volume gain and on the prevention of the progressive decrease in muscle mass with aging in combination with maintaining regular physical activity.

Abstract

Sedentary lifestyle and aging favor the increasing prevalence of obesity and type 2 diabetes and their comorbidities. The loss of lean body mass reduces muscle strength, resulting in impaired functional capacity and leading to increased risks of chronic diseases with advancing age. Besides aging, conditions such as inappetence, social isolation, and inadequate dietary intake cause the loss of lean body mass and increased abdominal fatty mass, resulting in sarcopenic obesity and predisposition to type 2 diabetes. Compared to younger people, this condition is more common in the elderly owing to natural changes in body composition associated with aging. Lifestyle changes such as increased physical activity and improved dietary behaviors are effective for preventing the occurrence of comorbidities. Regarding muscle nutrition, besides caloric adequacy, meeting the requirements for the consumption of dietary amino acids and proteins is important for treating sarcopenia and sarcopenic obesity because muscle tissue mainly consists of proteins and is, therefore, the largest reservoir of amino acids in the body. Thus, this review discusses the effects of dietary protein on the preservation of lean body mass, improvements in the functional capacity of muscle tissue, and prevention of chronic diseases such as type 2 diabetes. In addition, we address the effects of regular physical training associated with dietary protein strategies on lean body mass, body fat loss, and muscle strength in the elderly at a risk for type 2 diabetes development.

Keywords: Diabetes, obesity, aging, sarcopenia, protein, supplements, frailty, elderly, vitamin D, creatine, β-hydroxy β-methylbutyrate, acid leucine

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Introduction

Diabetes is one of the most prevalent diseases in the world, with the number of affected adults increasing from 108 million in 1980 to about 422 million in 2014, and it was the seventh leading cause of death in 2016. Almost half of all deaths attributable to high blood glucose occur before the age of 70.[1] Because diabetes is a chronic disease with high rates of comorbidities, besides its impact on public health, the resources necessary to control diabetes are costly for both the patients and their relatives.[1,2]

The urbanization process, including the modification of eating behaviors towards the consumption of unhealthy meals and increasingly sedentary lifestyle, and aging populations has resulted in an increased occurrence of obesity

and subsequent rise in the risk of diabetes and its comorbidities.[3–5] These conditions can be prevented by undergoing regular follow-ups with multidisciplinary teams and engaging in health action programs that include physical activity (exercise) and nutritional interventions aimed at lifestyle changes. These changes are intended to lead to the maintenance of mitochondrial function and its concentration in the skeletal muscle.[6]

Skeletal muscle comprises approximately 40% of human body mass, is responsible for locomotion, and is the largest site of utilization of glucose and fatty acids. Its regeneration is an integral part of the muscle's physiology; a failure in this process decreases contractile function, resulting in muscular atrophy, which is one of the complications of obesity and type 2 diabetes (T2D). Additionally, aging and sedentary lifestyle increase the oxidative stress of DNA, lipids, and proteins. These conditions increase inflammation and insulin resistance, contributing to the reduction of cytokines related to muscle nutrition, suppression of lipogenesis, and increase in energy expenditure.[7–9]

In our previous study, we observed that a three-month nutritional intervention associated with recreational soccer improved the physical capacity, dyslipidemic status, and insulin sensitivity and decreased markers of muscle catabolism in elderly patients with T2D.[8,10] In a recent research review including our study, we showed that nonconventional physical training such as recreational soccer in young and middle-aged women was effective in preventing and treating modern diseases, including hypertension, T2D, and osteopenia.[3] In a recently published special issue, we also showed that practicing a favorite sport two to three times per week for 1 h has many benefits that it is difficult to understand why we gave up this practice during our youth.[11]

Regarding nutritional assessment, generally, healthy meals with reduced portion sizes and low energy density result in weight loss and the prevention of chronic metabolic diseases.[10,12] Additionally, the population individuals with T2D consists particularly of those who are middle-aged and elderly with unhealthy lifestyles, in which the loss of lean body mass as a natural event in aging leads to sarcopenic obesity. However, long-term adherence to better lifestyle behaviors is common, which impairs the maintenance weight loss in the short term after a period of energy restriction.[13]

Thus, interventions that lead to weight loss and its maintenance result in more robust changes in the long-term, resulting in a better quality of life in the elderly besides decreased public health expenditures. In this context, the use of different types of protein supplements has been less mentioned in the nutritional guidelines applied as a strategy to attenuate the loss of lean body mass, improve the functional capacity of the muscle, ultimately control blood and clinical parameters, and prevent the occurrence of sarcopenic obesity, a common phenomenon in the elderly which can predispose them to T2D.[14,15] Furthermore, the elderly may benefit from exercise-induced increases in mitochondrial biogenesis and cellular antioxidant defense.[6,16] These functional aspects are rapidly reversed after the reduction or cessation of physical activity.[17–19]

Maintaining an acceptable level of exercise is vital in preserving muscle function with advanced age. Regarding single muscle fiber level, the muscles of healthy men over 70 years of age are protected to some degree by physical activity compared to sedentary controls in the same age group.[4] A decrease in muscle mass impacts the health of adults and the elderly, has important implications for excess weight gain, poses a risk for the development of insulin resistance and T2D, and impairs quality of life.[9]

Son *et al*.[20] first demonstrated the association between muscle mass and T2D risk in 2017. In their two-yearly prospective study of an Asian population, they reported that low muscle mass index (assessed by weight-adjusted skeletal muscle mass) was strongly related to the incidence of diabetes in middle-aged and older adults.

However, there is still a lack of randomized clinical trial studies that directly assess the association between increased muscle mass and the prevention of new cases of diabetes. Thus, this review discusses the effects of a more active lifestyle, including physical training of different types, intensities, and volumes, and the effects of protein supplementation as strategies for preventing muscle catabolism on the elderly population at a risk for developing T2D.

Muscle tissue changes in aging: Frailty, atrophy, and sarcopenia

The loss of muscle mass is mainly responsible for frailty in the elderly, which is characterized by weight loss, weakness, and slow walking and which contributes to health risk factors such as fracture, immobility, functional decline, and loss of independence. Frailty is a state of high vulnerability that leads to disability, dependence, and mortality due to decreased physical reserves in the elderly.[21]

Frailty has five components, namely: exhaustion, physical inactivity, muscle weakness, slow walking speed, and weight loss. Two of these components, muscle weakness and slow walking speed, are characteristic of sarcopenia, which is generally prevalent in the elderly.[5] Unintentional sudden weight loss; frequent falls; inability to perform activities of daily living; hospitalization; and the presence of chronic diseases such as T2D, cardiovascular disease, chronic obstructive pulmonary disease, rheumatoid arthritis, and cancer are associated with sarcopenia.[22,23]

The diagnostic criteria for sarcopenia were approved in 2010 by the *European Working Group on Sarcopenia in Older People*, which associated sarcopenia with other syndromes such as cachexia, frailty, and obesity.[24]

Makizako *et al*.[12] examined 10,092 elderly individuals over 65 years of age using handgrip strength, five-time sit-to-stand, and walking speed tests. They reported a negative correlation between physical performance and aging among both elderly women and men. The reduction in handgrip strength was observed more with aging among elderly men, while decreased walking speed was more commonly observed with aging in elderly women. Assessment of anthropometry profiles with age revealed

increased body mass index (BMI) and fat mass with decreased muscle mass in both sexes, which were more pronounced in women. These changes suggested a direct association between body composition and physical activity in the elderly.

Sarcopenic obesity: Origin and impact on health

Aging induces changes in body composition such as increased visceral fat and reduced muscle mass.[12,25,26] Therefore, the new concept of sarcopenic obesity has emerged, which reflects the combination of sarcopenia and obesity.[8]

Sarcopenic obesity was first defined as the coexistence of sarcopenia and obesity in one individual based on a low amount of muscle tissue (sarcopenia) and excessive adipose tissue.[27]

The current interpretation of sarcopenia combines muscle tissue reduction, low muscle strength, and low physical capacity, although the clinical explanation of sarcopenia varies globally; thus, the definition of obesity is related to the BMI of an individual.[28]

Sarcopenic obesity is more prevalent in the elderly than in adults due to alterations in body composition related to advanced age.[29]

Evidence has shown that the elderly with sarcopenic obesity have higher risks of mobility,[30] metabolic diseases, hypertension, cardiovascular diseases, and mortality.[31] Vaitkus and Celi[32] reported that increased adipose tissue is associated with the development and progression of cancer-associated cachexia, which along with other metabolic and chronic diseases is associated with higher health care costs.[31,32] The clinical problems associated with sarcopenic obesity are much higher when compared to those for sarcopenia or isolated obesity,[33] suggesting that these conditions have independent adverse and additives effects in the health of the elderly.

The US Foundation for the National Institutes of Health Sarcopenia Project (FNIHSP) was the largest sarcopenia project, including data from nine studies with participants over 65 years of age. The data reflect diversity regarding races, ethnicities, regions, and health status. After screening for physical function tested by gait speed and strength assessment using handgrip strength and measurement of lean body mass by dual-energy X-ray absorptiometry (DXA), the FNIHSP assigned a gait speed less than or equal to 0.8 m/s as indicative of poor physical function[34]. The suggested cut-off points for muscle weakness were handgrip strengths of less than 26 kg for men and less than 16 kg for women. Regarding muscle mass assessment, experts in the study preferred a measure that accounted for BMI instead of height, which was suggested earlier in other sarcopenia groups. The recommended cut-off points for appendicular lean mass (ALM) using DXA and BMI were less than 0.789 for men and less than 0.512 for women.[25]

The current definitions of sarcopenic obesity were developed primarily based on data from multicenter studies, and, until now, there is no universal consensus regarding these definitions. According to Kemmler *et al.*,[34] the incidence of sarcopenia and sarcopenic obesity is steadily increasing, which is detrimental to the aging population; however, different definitions, components, and cut-off points make it difficult to accurately assess their prevalence.

Therefore, longitudinal studies and randomized clinical trials should be conducted to investigate the causes and prognoses of sarcopenic obesity to provide prevention and treatment strategies based on clinical and scientific evidence.

Protein nutritional support in aging

Adequate caloric intake is an important determinant of health status, especially when degenerative conditions become a predominant risk in the treatment of diseases, as is the case for aging. The maintenance of nutritional status is the best measure to counteract the risk of protein-caloric malnutrition and its complications, which often affect the elderly population, particularly frail patients.[35]

The nutrients most consistently associated with sarcopenia and sarcopenic obesity are proteins and (essential) amino acids because muscle tissue consists primarily of protein and is the largest reservoir of amino acids in the body. Epidemiological studies have demonstrated the association between inadequate protein intake and poor physical performance in aging.[36]

Several studies and consensuses on the standard of care have stated that protein intake above 0.8 g/kg/d may result in greater muscle health benefits than the recommended dietary allowance (RDA).[35,37–39] According to Bassil *et al.*,[9] a protein intake of 1.0–1.2 g/kg/d is recommended for the preservation of healthy muscles in aging, while 1.2–1.5 g/kg/d of protein may be necessary in elderly with acute or chronic diseases.

However, according to the Standards of Medical Care in Diabetes,[40] no evidence supports the assumption that adjusting the daily level of protein intake (typically 1–1.5 g/kg body weight/day or 15–20% of total calories) will optimize either glycemic control or cardiovascular disease risk and that protein intake goals should be individualized based on the current eating patterns. For individuals with diabetic kidney disease (with albuminuria and/or reduced estimated glomerular filtration rate), dietary protein levels should be maintained at an RDA of 0.8 g/kg body weight/day. Reducing the amount of dietary protein below the RDA is not recommended because it does not alter glycemic measures, cardiovascular risk measures, or the rate at which the glomerular filtration rate declines; moreover, it may contribute to the depletion of lean body mass.[40]

In a pilot study by Sammarco *et al.*,[39] middle-aged and elderly women with a diagnosis of sarcopenic obesity undergoing a nutritional program for weight loss showed improvements in muscle strength and preservation of muscle tissue with a low-calorie, high-protein diet (1.2–1.4 g/kg/day hypocaloric diet plus an additional 15 g of protein supplements) compared to those with a low-calorie diet.

In a double-blind randomized design study, Dirks *et al.*[41] demonstrated the impact of protein supplementation (30 g/day) on increasing lean body mass following resistance training in 34 fragile elderly subjects. After 24 weeks of treatment, hypertrophy was observed in 23% and 34% of type I and II muscle fibers, respectively ($P < 0.01$), in the supplemented group.

Table 1 presents a compilation of 12 clinical trials published in 2016, 2017, and 2018, which were identified in the PubMed database using the following search terms: protein supplementation, sarcopenia, and elderly. This material was organized after a previous search revealed 178 articles published until the present date. Manuscripts published before the cut-off period, as well as untested clinical trials, reviews, and meta-analyses, were excluded. Hence, this selection evaluates only published clinical trials within the last three years with planned interventions in humans.

The clinical findings reported in Table 1 indicated that protein supplementation in the elderly increased muscle mass,[39,42–47] muscle strength,[14,39,44,48–50] type I and II muscle fibers,[41] body fat loss (range, 0.62 kg–0.9 kg),[47] quadriceps strength,[42] and physical performance.[42,46,49] Even with variations in dietary supplementation protocols, these findings demonstrate that interventions with exercise and protein supplementation stimulate muscle anabolism and fat loss in the elderly.

Proteins: Type and quantity

The body's proteins are constantly being transferred. This constant synthesis and degradation of proteins provides a mechanism for maintaining protein in the case of damage related to oxidative stress, protein malformation, or other processes.[51] The synthesis and degradation of proteins in the human body are affected by several variables, including age, physical activity, sex, hormones, diseases, and diet.[52]

The loss in protein body content, mainly measured as a reduction in lean body mass, occurs due to acute and chronic imbalances in protein turnover.[53]

Protein intake should ensure a balanced provision of dietary amino acids. The content, digestibility coefficients, and relative proportions of amino acids in dietary proteins are the determinants of its nutritional value. Amino acids are essential precursors for the synthesis of proteins, peptides, and low-molecular-weight substances with enormous physiological importance. A sufficient supply of both essential amino acids and amino acids that are synthesizable de novo in animal cells (AASAs) plays a key role in sustaining skeletal muscle mass and function, while improving insulin sensitivity, ameliorating aging-associated sarcopenia, and reducing white-fat accretion.[54,55]

Protein requirements for healthy adults and the elderly are established according to the RDA recommendations and currently range between 0.8 and 1 g/kg/d.[56] RDA guidelines have been widely used in nutritional practice as a target for macronutrient intake[51] and its approach involves a minimum requirement rather than a recommended intake of protein; thus, it must be determined whether the best health outcomes could be achieved with protein intakes higher than those recommended by the RDA.[57]

According to a report by the Institute of Medicine,[56] current protein requirements set the RDA within the previously reported values based on nitrogen balance. Nitrogen balance has been used for over 60 years to establish protein recommendations. However, nitrogen deficiency deficits have been recognized, despite the fact that this methodology supports the determination of the RDA for proteins.

The current Food and Agricultural Organization of the United Nations/World Health Organization (FAO/WHO) and Dietary Recommended Intake (DRI) recommendations for protein intake in healthy elderly individuals are based on nitrogen balance data derived from studies conducted primarily in healthy young adults. The estimated average requirement and RDA are 0.66 and 0.8 g/kg/d, respectively.[58]

The elderly suffer a higher prevalence of chronic disease than do younger adults; a recent working group has used this as evidence for recommending a protein intake of 1.2–1.5 g/kg/d for the elderly. This recommendation is supported by epidemiologic research showing a positive association between higher dietary protein intake and fewer health problems in the elderly, leading to a beneficial effect of protein intakes higher than the current recommendations.[58]

According to Phillips,[51] it is important to emphasize that even these estimates may not be optimal and should be tested in long-term trials to establish the ramifications of this consumption and recommendation.

The relevance for long-term health outcomes in the elderly is impacted by the intake of at least 1.2 g/kg/d of proteins for the maintenance of muscle tissue. This point is even more relevant if we consider that 25% of elderly men and up to 50% of elderly women do not even consume the RDA-recommended protein intake of 0.8 g/kg/d.[59]

It is recommended that the RDA for proteins be derived from protein sources of high biological value. Protein quality has been estimated using the protein digestibility-corrected amino acid score (PDCAAS). There is a specialized recommendation that PDCAAS should be replaced with a new scoring system, the digestible indispensable amino acid score (DIAAS). These scores differ in that the PDCAAS uses fecal protein digestibility, and there is significant bacterial (colonic) metabolism of amino acids that can falsely enhance the true protein digestibility values, while the DIASS is based on the effective digestibility of the proteins in the gut. However, the DIAAS requires more evaluation in clinical studies.[51]

According to Wu,[54] both the PDCAAS and DIAAS indexes ignore the physiological needs for amino acids essential for tissue protein; for example, skeletal muscle, synthesis or function, and the important roles of non-essential amino acids in human nutrition and metabolism. An adequate intake of essential and non-essential amino acids can ensure the maintenance of muscle tissue, thereby stimulating the oxidation of energy substrates (including fatty acids and glucose) in the adipocytes, liver, skeletal muscle, and heart and assisting in the reduction of obesity.

Table 1. Characteristics of randomized controlled trials investigating the effect of protein supplementation in older adults.

Types of supplements	Amounts	Duration	Target population	Sample size	Results	Study design	Authors
Oral protein dietary supplement with 187 kcal – (20% protein/60% carbohydrate/20% fat). Enriched with 1.8 g leucine, omega-3 and vitamin D.	125 mL of supplement 3× /day	4 month	Patients with DPOC[a](51% masc) de 43–80 years	81 participants	**Supplemented group vs. placebo** +0.6 kg muscle mass vs. +0.3 kg muscle mass +13.6 Nm quadriceps strength vs. +10.8 Nm +144 s cycle endurance time vs. +244.5 s	Randomized, double-blind, placebo-controlled	Bool *et al.*[42]
HMB[b] oral dietary supplement enriched with vitamin D, carbohydrates, proteins and fats	Oral dietary supplement of HMB 2 bottles/day – (220 mL × 2, total: 660 kcal) – 1.5 kcal/mL, 24% protein; 29% fat and 46% carbohydrates. Enriched with HMB 0.7 g/100 mL, 25 (OH) D 227 IU/100 mL	Mean of hospitalization (42 days +/− 20.9/days)	Patients with hip fracture older than 65 years	107 participants	**Supplemented group vs. control group** 1.2 kg strength vs. = strength =muscle mass vs. – 0.8 kg muscle mass	Randomized multicenter randomized	Malafarina *et al.*[48]
Hypocaloric diet plus placebo (A) and hypocaloric high-protein diet (1.2–1.4 g/kg body weight reference/day) (B).	Amount of Protein (1.2–1.4 g/kg body weight reference/day) + 15 g leucine	4 month	Women 41–74 years	18 participants	**Group A vs. Group B** =Muscle strength vs. +1.6 kg muscle strength −1.3 kg lean body mass vs. −0.5 kg lean body mass	Randomized controlled	Sammarco *et al.*[39]
Whey protein	2 × 15 g/day	24 weeks	Men and Women of approximately 77 years	34 participants	**Supplemented group vs. control group** +23% CSA[c] type I fibers vs.=CSA type I fibers +34% CSA type II fibers vs. +20% CSAtype II fibers	Randomized, double-blind, placebo-controlled with 2 arms in parallel	Dirks *et al.*[41]
Whey protein	0.4 g/kg protein weight per meal	24 weeks	Men and women 50–70 years old	60 participants	**Supplemented group vs. control group-** + 0.7 kg muscle mass vs. −0.2 kg muscle mass	Randomized controlled	Norton *et al.*[43]
Whey protein with leucine and vitamin D	22 g (essential amino acids = 10.9 g, including 4 g of leucine and vitamin D [2.5 μg (100 IU)])	12 weeks	Men and Women 80.3 years (average)	130 participants	**Supplemented group vs. placebo group-** + 1.3 kg fat free mass vs.−0.3 kg fat free mass + 3.2 kg handgrip strength vs.-0.47 kg handgrip strength +0.42 kg/m^2 BMI vs. −0.42 kg/m^2 BMI	Randomized, double-blind, placebo-controlled	Rondanelli *et al.*[44]

(continued)

Table 1. Continued

Types of supplements	Amounts	Duration	Target population	Sample size	Results	Study design	Authors
Supplement of protein with carbohydrate and vitamins	20.7 g of protein + 9.3 g of carbohydrate + 3 g of fat + vitamins (800 IU of vitamin D, 2.9 mg of vitamin B6, 3 μg of vitamin B12)	6 months	Women of 83.6 years (average)	91 participants	RT[d] vs. RTS[e] +14%MQ* vs. +12%MQ*; +18% performance vs. 15% performance	Randomized controlled	Hofmann *et al.*[49]
PB[f]: (25% soy, 25% whey protein and 50% casein) , WPI[g] (whey protein isolate)	30 g/day	1 h after the leg extension exercise (8 sets of 10 repetitions with 70% RM)	Men 55 to 75 years old	20 participants	**WPI vs. PB** Group WPI:+mTORC1 Group PB:+ mTORC1 signaling and muscle protein synthesis Both WPI and PB increased muscle mass	Randomized, double-blind, controlled	Borack *et al.*[22]
Nutritional supplement	Nutritional supplement (150 kcal, 20 g of whey protein, 800 IU of vitamin D, 119 mL of beverage)	6 months	Men and women 78.5 years (average)	149 participants	**Supplemented group vs. placebo group** −0.62 kg body fat vs. −0.9 kg body fat; +3.03 cm^2 muscle area vs. −1.51 cm^2 muscle area of the thigh; −0.6 cm^2 intramuscular fat vs. −0.3 cm^2 intramuscular fat	Randomized, double-blind, placebo-controlled	Englund *et al.*[47]
Whey protein	Whey protein with 22.3 g protein	24 weeks	Women 65–80 years	81 participants	EX[h]+PRO[i] group vs. EX group vs. PRO group +1.1 kg Body weight (BW) vs. +0.5 kg BW vs. −0.2 kg BW +0.5 kg LL[j] vs. +0.3 kg LL vs. +0.1 kg LL; +2.6 kg knee extension strength vs. +1.1 kg knee extension strength vs. −1.2 kg knee extension strength	Randomized controlled trial	Mori and Tokuda[14]
Milk plus soy protein	25 g of soy chocolate-flavored	16 weeks	Menopausal women	32 participants	Soy+RT[d] vs. placebo+RT[d]: +1.1	Randomized and placebo-controlled	Orsatti *et al.*[46]

(continued)

Table 1. Continued

Types of supplements	Amounts	Duration	Target population	Sample size	Results	Study design	Authors
	powderadded to 200 mL of milk				kg muscle mass vs. +1.5 kg muscle mass, +12.5 kg of muscle strength vs. +6.7 kg of muscle strength, +6.7 kg knee extension vs. + 3.7 kg knee extension, +24.2 kg total load vs. +15.1 kg total load	(placebo group vs. experimental group	
Whey protein	24 g of whey protein per day	24 weeks	Men and women 60–93 years	47 participants	**Supplemented group vs. control group** +30%, grip strength vs. +2,1% grip strength +42,7% knee extensor force vs. +12,9% knee extensor force +34,6% gait speed vs. +40% gait speed:	Double Blinded, Randomized Controlled Trial	Nicolli *et al*.[50]

[a]aDPOC: chronic obstructive pulmonary disease; [b]HMB: Beta-hydroxy-betamethylbutyrate; [c]CSA: cross sectional area; [d]RT: resistance training; [e]RTS: resistance training supplementation; [f]PB: protein blend; [g]WPI: whey protein isolate; [h]EX: exercise; [i]PRO: protein supplementation; [j]LL: Lower limb muscle mass; (+) increase; (−) decrease; (=) similar

A conditionally essential amino acid in adult mammals is L-arginine, a precursor for the synthesis of biologically important molecules.[60] Physiological levels of arginine and NO promote fat oxidation and decrease fat synthesis in a tissue-specific manner, thus representing a useful strategy to reduce obesity, improve insulin sensitivity, and improve health in T2D subjects.[60,61]

In humans, the supplementation of 30 g of arginine/day for 90 days was tolerable and reduced systolic blood pressure and serum glucose concentration in women and serum free fatty acids levels in both men and women.[61] Furthermore, arginine activates the mammalian target of rapamycin (mTOR) cell signaling pathway and therefore protein synthesis in skeletal muscle.[62]

Protein supplementation is a strategy for adequate protein intake. Whey protein (milk serum) mainly combined with exercise has been suggested to be beneficial for the elderly in maintaining or increasing lean body mass and improve health parameters. Compared to other sources of proteins, whey protein contains high levels of branched-chain amino acids (BCAAs), especially leucine, an amino acid involved in stimulating protein synthesis.[63]

Whey protein is rapidly digested, therefore resulting in a rapid increase in blood amino acid concentrations.[64] Casein, another milk protein, has a slower digestion profile than that of whey protein. As a result, casein prolongs hyperaminoacidemia compared to milk serum. Although the increase in blood amino acid levels after ingestion of casein does not reach the concentrations observed with whey intake, protein synthesis remains activated several hours post-exercise.[65] Milk is not the only source of protein supplementation. Soy also contains many antioxidants and is a good alternative source of protein for those on a vegetarian diet.[66]

Clinical trials conducted by Borack *et al.*[22] assessed the ingestion of approximately 30 g/day of whey protein, casein, and soy protein (all containing approximately 3 g of leucine) in the elderly compared to an intervention without supplements. Similar responses were observed for all types of supplementation, whereas in hyperaminoacidemia, the leucine and isoleucine levels were twice those at baseline, reaching more than 100 mmol/L 2 h post-ingestion. Regarding protein synthesis signaling, mTORC1 phosphorylation was significantly increased at 2 and 4 h post-ingestion at three times the baseline values. These data add additional evidence for the use of whey or soy milk as targeted nutritional interventions to treat sarcopenia.

Nutritional support in aging (vitamin D, HMB, leucine, and creatine)

Creatine

Although aging is an inexorable matrix process, malnutrition and physical inactivity are well-known factors that may aggravate impaired muscular function in the elderly.[67]

Creatine supplementation has emerged as one of the few effective dietary interventions capable of attenuating aging-related declines in muscle and cognitive function.[68]

Recent findings confirm the important therapeutic effects of creatine supplementation and demonstrate the following improvements in the elderly: increased muscle strength and resistance to fatigue, improved performance of daily activities, and avoidance of bone loss.[51]

Creatine plays an important role in providing fast energy and is also used by the brain to increase mental performance. It is stored mainly in the skeletal muscles (90%) as phosphocreatine, a high-energy phosphate involved in the rapid re-synthesis of adenosine triphosphate during muscle contraction.[69]

Pinto *et al.*[69] observed a significant increase in lean body mass in the group supplemented with 5 g of creatine/day and resistance exercise at the 12-week follow-up ($+0.9 \pm 1.2$ kg, $P = 0.014$) than placebo and resistance exercise group.

Another randomized, double-blind trial conducted by Evans *et al.*[67] reported significant results in elderly participants administered L-carnitine, creatine, and leucine supplements containing L-carnitine (1500 mg), L-leucine (2000 mg), creatine (3000 mg), and vitamin D3 (10 μg) for eight weeks. This compound showed an increase in total lean body mass of 1.0 kg ($P = 0.013$) compared to that at baseline.

The effects of creatine supplementation on glycemic control of patients with T2D were evaluated by Gualano *et al.*[68] In this randomized, double-blind study, patients received 5 g/day of creatine, or placebo, for 12 weeks during a physical training program. Glycated hemoglobin (HbA1c) and glucose (ΔAUC) levels were significantly lower in the creatine group than in the placebo group. In the creatine group, lower blood glucose levels were observed at 0, 30, and 60 min, during a meal tolerance test. Moreover, an increase in GLUT4 translocation to the sarcolemma was observed in the muscles of T2D patients. Thus, creatine supplementation may result in lean body mass gain, improving the muscular function of the elderly, in addition to contributing to glycemic control; thus, it may be useful as a therapy in patients with T2D.[68]

Vitamin D

Elderly patients, mainly those older than 85 years of age, are at the highest risk for low vitamin D levels, which influences the loss of muscle mass and strength and physical decline.[70]

Moreover, inadequate dietary intake, low physical activity levels, and smoking lead to the loss of muscle mass in the elderly. In particular, low intakes of vitamin D, proteins, and antioxidant nutrients (carotenoids, selenium, and vitamins E and C) have been associated with sarcopenia and frailty in the elderly.[24]

The reported positive effects of vitamin D supplementation in the elderly include increased muscle function and strength and reduced falls.[71–74]

In a prospective, large-scale observational study, Oliveira *et al.*[72] examined volunteers >50 years of age and investigated the serum concentrations of 25(OH)D and three inflammatory markers (plasma fibrinogen, blood cell count, and C-reactive protein [CRP]). Positive correlations were observed between low serum vitamin D

concentrations and increased concentration of inflammatory markers, indicating the association of vitamin D as an immunosuppressive agent.

In the study by Apaydin *et al.*,[74] quadriceps and hamstring muscle strength in postmenopausal women increased significantly after 4 and 12 weeks, respectively, of daily oral dosages of 800 IU vitamin D supplementation when compared to those who follow a single oral dosage (300 UI). Although the daily dosages were more effective in improving muscle strength, the single administration of vitamin D was more effective in increasing vitamin D levels.

Granic *et al.*[70] classified quartiles (SQ1–SQ4) of vitamin D concentration during the four seasons of the year (summer, autumn, winter, spring), with SQ1 the lowest and SQ4 the highest vitamin D values to assess grip strength and decline in physical performance in 845 elderly people over 65 years of age over five years. They observed an association between reduced grip strength and lower vitamin D levels among the participants, particularly in men. The decline in physical performance did not differ between seasons and was similar among participants.

Gmiat *et al.*[73] evaluated the association of vitamin D supplementation and 1 h walking in elderly women with high (HVitD) or low (LVitD) serum vitamin D levels (less than 20 ng/mL) for 12 weeks. The HVitD group had lower concentrations of proinflammatory markers such as the high-mobility group box 1 (HMGB1) and interleukin-6 (IL-6). The vitamin D supplementation improved serum levels in both LVitD (40.98 ± 14.0 ng/mL) and HVitD (41.25 ± 26.3 ng/mL) groups. The training program was generally effective in reducing the concentrations of inflammatory markers, demonstrating that vitamin D is related to decreased levels of proinflammatory cytokines and muscle function. Significant negative correlations were observed between irisin and HMGB1 in both groups both before and after the intervention. Furthermore, the relationship between these proteins was higher in the HVitD group and increased with physical training. Conversely, in the LVitD group, the higher the irisin level, the lower the HMGB1 level.

In summary, the number of studies on vitamin D studies is increasing largely because it is reported to be deficient in the elderly and because of its association with loss of muscle mass and strength, highlighting the importance of sun exposure, the intake of foods rich in vitamin D such as lean dairy products, and regular physical training.

HMB

HMB, a metabolite derived from leucine that is produced in the skeletal muscle, is an essential amino acid supplement. It has been evaluated as a nutritional supplement to enhance muscle protein synthesis in healthy or frail elderly subjects since it decreases muscle catabolism through the modulation of the ubiquitin-proteasome pathway.[75,76]

According to Holeček,[75] HMB supplementation increases mitochondrial biogenesis and fat oxidation, attenuates muscle damage caused by strenuous exercise, contributes to muscle hypertrophy, increases aerobic performance, and reduces fatigue resistance, thus contributing to the treatment of elderly patients with sarcopenia.

Kuriyan *et al.*[77] compared plasma HMB concentrations in approximately 300 individuals at different stages of life (children, adults, and elderly) with lean body mass and handgrip strength. The plasma concentration of HMB in children was higher compared to those in adults and the elderly. Regarding sex, women had lower plasma HMB levels than those in men. An inverse association between age and endogenous HMB concentration was observed, with concentrations of 176.5, 159.7, and 154.1 ng/mL in children, adults, and the elderly, respectively. Significant associations between HMB plasma concentrations and handgrip strength and lean body mass were not observed in children, while a positive association was observed in adults and the elderly.

Berton *et al.*[78] investigated the association between the supplementation of 1.5 g of HMB for eight weeks and physical performance based on changes in short physical performance battery score as the primary outcome and changes in peak torque, isometric and isokinetic strength of the lower limbs, 6-min walking test, handgrip strength, and endurance as the secondary outcomes. Body composition was assessed by DXA in elderly women ($>$ 65 years) undergoing a low-intensity physical activity program twice weekly. After eight weeks, no significant difference in body composition was observed between the control and supplementation groups. Physical performance improvements were observed for all exercises except for handgrip strength in the supplementation group. Therefore, even at low doses, HMB supplementation can improve physical performance in the elderly.

Conversely, the meta-analysis by Sanchez-Martinez *et al.*[79] included six randomized clinical studies performed with athletes aged between 19 and 25 years who were administered HMB supplementation ranging from 3 to 6 g/day or placebo. No significant differences in muscle strength (leg press and supine) and body composition (fat mass, muscle mass, and body mass) were observed in the HMB group compared to those in the placebo group. It is important to note that this study considered both different modalities and different intervention times ($\leq$ 4 weeks and $\geq$ 4 weeks).

Gentles and Phillips[80] reported discrepancies in several publications[81–83] that investigated the action of HMB-free acid (HMB-FA) supplementation for 12 weeks in combination with resistance and aerobic exercise in young men. These articles were published based on the results of a study reporting a positive ergogenic effect in the supplementation group vs. the placebo group, with improvements in muscle strength and muscle mass. However, many inconsistencies in these studies have been described, as an example, the placebo group presented in all three article is formed from the same subjects, in which the numbers in the control groups differ without explanation.[79]

Although a few findings suggest that HMB supplementation results in ergogenic effect, in some studies the effect size was small. Clinical studies in humans are lacking, and even human studies have biases associated with methodology, samples, time, and therefore more high-quality

randomized clinical trials should be carried out with greater methodological rigor.

Leucine

Leucine is an essential amino acid that is not synthesized by the body and which is ingested through diets rich in meat, fish, soy, nuts, and dairy products.[84] It is one of the BCAAs that increases insulin secretion and influences molecular regulation of muscle protein synthesis (via the mTOR pathway), thereby stimulating anabolism, provided a sufficient supply of essential amino acids to the muscles.[42]

Leucine affects directly the activation of the mTOR complex signaling pathways; however, its action of skeletal muscle anabolism remains unknown. One likely mechanism of sarcopenia is the anabolic resistance of older muscle to nutritional stimulation; supplementation of leucine could decrease this muscular catabolism.[45]

In the study by Murphy *et al.*,[85] older men were randomly assigned to receive low- (0.8 g/kg/d) or high- (1.2 g/kg/d) protein diets while ingesting a placebo or 5 g leucine/meal and performing resistance exercise during treatment period. Leucinemia was higher with leucine treatment than with placebo administration. Muscle protein synthesis was similar in the low- and high-protein diets but higher with leucine treatment, demonstrating the need for isolate leucine supplementation in diets with low or restricted protein intake because of comorbid conditions or in those who consume adequate protein, particularly when combined with resistance training.

Mitchell *et al.*[86] evaluated the effect of leucine after supplementation of 15 g of essential amino acids in elderly individuals. Apart from the pool of amino acids, they also received an additional 3 g of leucine 90 min after the meal. Analysis of blood and biopsy skeletal muscle samples collected post-intervention revealed that oral essential amino acids resulted in rapid insulinemia, essential aminoacidemia, and activation of mTORC1 signaling. Phosphorylation of mTORC1 substrates returned to fasting levels at 240 min post-feed in both groups. Therefore, oral leucine intake after adequate doses of essential amino acids did not result in changes in skeletal muscle anabolic signaling or protein synthesis.

As reported by Borack and Volpi,[22] consistent leucine supplementation increases muscle protein synthesis but is not associated with improvements in muscle gain.

Although leucine is showed to influence the activation of mTOR and muscle protein synthesis, isolate supplementation does not result in muscle adaptation and, thus, may not provide a benefit to muscle accretion in older adults.

Conclusion

Based on the current literature review, we have drawn the following conclusions and considerations for future research.

Aging leads to slowed movements and inappetence, thus inducing changes in body composition such as increased visceral fat and progressive loss of muscle mass, strength, and functional capacity, leading to the occurrence of sarcopenic obesity and predisposing elderly to the development of chronic diseases such as obesity and T2D.

Exercise may decrease the deleterious effects of aging and modulate the molecular mechanisms activated in T2D, thus favoring atrophy/sarcopenia and insulin resistance in skeletal muscle. In addition to exercise, protein supplementation strategies aim to increase or preserve muscle mass in aging. Thus, exercise and adequate caloric and protein intake are the main pillars in anabolism. Protein, arginine, leucine, HMB, and creatine supplementation may be indicated for increased muscle mass, strength, and volume and may improve the functional aspects of the muscle in the elderly, particularly those with frailty and malnutrition and in sedentary patients with chronic metabolic diseases such as T2D. However, depending on the patient's clinical and nutritional status, such as renal and hepatic dysfunction, protein metabolism capacity can reach its peak with protein supplementation, in which case dosage reduction or withdrawal of the supplementation may be necessary. With the increasing popularity and marketing of nutritional supplement industries, it is important to evaluate the efficacy of ergogenic effects, particularly in the elderly and chronic metabolic disease populations.

Further research is necessary to investigate the impact of different types of exercise on systemically improving metabolic outcomes and decreasing frailty with aging. From the molecular point of view, evaluating the impact of a proper lifestyle on insulin sensitivity and the role of improved muscle anabolism for protection against the development of metabolic chronic disease is of vital importance. Such efforts will aid in the identification of patients with early onset of insulin resistance and mitochondrial dysfunction or muscle atrophy induced by aging and who are at risk of metabolic diseases. This stratification will allow for a personalized prescription of exercises that may be beneficial to avoid T2D and secondary aging.

Regarding nutrition, the daily therapeutic dose of proteins and amino acids to decrease the loss of muscle mass with aging, particularly for the prevention or control of T2D and its comorbidities, is another challenge requiring further investigation.

Long-term longitudinal clinical studies are necessary to further unravel the impact of exercise and nutrition on the mechanisms underlying sarcopenic obesity to lower the risk of T2D.

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ORCID iD

Maysa Vieira de Sousa https://orcid.org/0000-0003-2211-2209

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