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REVIEW ARTICLE

Dietary protein supplementation in the elderly for limiting muscle mass loss

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Abstract Supplementation with whey and other dietary protein, mainly associated with exercise training, has been proposed to be beneficial for the elderly to gain and maintain lean body mass and improve health parameters. The main objective of this review is to examine the evidence provided by the scientific literature indicating benefit from such supplementation and to define the likely best strategy of protein uptake for optimal objectified results in the elderly. Overall, it appears that an intake of approximately 0.4 g protein/kg BW per meal thus representing 1.2–1.6 g protein/kg BW/day may be recommended taking into account potential anabolic resistance. The losses of the skeletal muscle mass contribute to lower the capacity to perform activities in daily living, emphasizing that an optimal protein consumption may represent an important parameter to preserve independence and contribute to health status. However, it is worth noting that the maximal intake of protein with no adverse effect is not known, and that high levels of protein intake is associated with increased transfer of protein to the colon with potential deleterious effects. Thus, it is important to examine in each individual case the benefit that can be expected from supplementation with whey protein, taking into account the usual protein dietary intake.

Keywords Whey protein · High protein diet · Anabolic resistance · Protein turnover · Sarcopenia · Aging

Abbreviations

LBM	Lean body mass
MPB	Muscle protein breakdown
mTOR	Mammalian target of rapamycin
MPS	Muscle protein synthesis
IGF-1	Insulin growth factor 1
p70S6k	Protein kinase p70S6
WPI	Whey protein
EAA	Essential amino acids
BCAA	Branched-chain amino acids
FSR	Fractional synthesis rate

Introduction

The human body is in a constant process of protein synthesis and degradation, and this metabolic cycle can be influenced by many factors, including physical activity, caloric deficit and senescence. The rate of renewal is about 300 g of protein per day (Lancha and Pereira-Lancha 2012), representing approximately 2% of the total protein content in adult man weighing 70 kg. Around 40% of body protein are contained in skeletal muscle, and their turnover is considered as relatively slow when compared with the

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splanchnic area turnover (Wall and van Loon 2013). The protein turnover is characterized by the balance between muscle protein synthesis (MPS) and muscle protein breakdown (MPB). Resistance training combined with adequate protein intake results in a positive protein turnover, with values of MPS being higher than MPB, leading then to muscle accretion (Cermak et al. 2012). In contrast, caloric restriction periods and/or senescence appears to increase the levels of MPB and lower MPS, resulting in a negative protein turnover and consequently muscle wasting (Areta et al. 2014; Barkoukis 2016; Phillips 2009). Over the time, the human muscle mass is dictated by the changes in MPS and MPB (Deutz and Wolfe 2013; Phillips 2004; Rennie et al. 2004).

To promote a positive protein turnover, it is necessary to take into account the quality of the protein source. Protein derived from dairy foods are considered as among the best in terms of the quality of protein sources (Hartman et al. 2007; Wilkinson et al. 2007). Regarding this latter aspect, whey protein is a fraction (~20%) of the milk protein, which is characterized by a high biological value notably because of its high content in essential amino acids, and particularly high leucine concentration (Pennings et al. 2011a). Leucine has been shown, at least in some cells and animal models, to be able to stimulate protein synthesis through the signaling pathway of the mammalian target of rapamycin complex 1 (mTORC1) (Churchward-Venne et al. 2014a; Dickinson and Rasmussen 2011; Farnfield et al. 2012; Layman et al. 2015). This signaling pathway is responsible for promoting changes in transcription of genes involved in protein synthesis, hence resulting in muscle remodeling (Dickinson and Rasmussen 2011; Farnfield et al. 2012; Fernandes et al. 2008). The mTORC1 is regulated by several cell signaling processes, which can be influenced by many external factors including amino acid availability and physical activity (mainly resistance training) (Churchward-Venne et al. 2014a; Daly et al. 2014; Dickinson et al. 2011; Dickinson and Rasmussen 2011; Kimball and Jefferson 2006; Farnfield et al. 2012). Interestingly, it appears that leucine can interact and stimulate mTORC1 in a different manner than resistance training (Duan et al. 2015; Farnfield et al. 2012; Wolfson et al. 2016; Saxton et al. 2016). Thus, an interesting strategy may be to combine these two stimuli, namely resistance training and leucine availability, to promote protein synthesis through dedicated signaling pathways. This increased stimulation of MPS may result in a positive protein turnover, which can contribute to the maintenance of skeletal muscle mass and possibly for muscle hypertrophy (Churchward-Venne et al. 2014a; Farnfield et al. 2012). Therefore, it is of paramount importance to understand the mechanisms involved in the stimulation of the mTORC1 signaling pathway, since this pathway is partly responsible for the muscle anabolism (McGlory and Phillips 2015).

The present review aimed at presenting current evidence suggesting that whey and other dietary protein supplementation may exert some beneficial effects in different subpopulations, contributing to the maintenance of skeletal muscle mass in the elderly. Since the upper level of protein consumption with no adverse effect is not known, we also discuss whether dietary protein, when consumed in excessive amounts, may cause some deleterious effects in the large intestine and kidney. To this end, a literature review was conducted using the scientific databases PubMed and Scholar Google.

Dietary protein characteristics

Given the well-known important role of amino acids not only as substrates for protein synthesis, but also as involved in various physiological and metabolic functions, the choice among the different sources of alimentary protein, with different amino acid content and digestibility, represents an important aspect to choose optimal dietary protein for effective effect on skeletal muscle physiology. Regarding this latter point, the whey protein can theoretically guarantee the supply necessary for the stimulation of MPS (Burd et al. 2012; Devries and Phillips 2015; Farnfield et al. 2012; Tang and Phillips 2009). Compared with soy protein, casein and collagen, whey protein has higher relative amount of EAA and leucine. Furthermore, amino acids derived from soy protein for instance appears less bioavailable than amino acids derived from casein and whey protein (Devries and Phillips 2015). In addition, whey protein can release biologically active peptides, which facilitate intestinal absorption (Meisel and Bockelmann 1999). When comparing whey protein with soy protein or whole milk, there is a higher rate of amino acid and oligopeptide absorption in the intestinal tract from the whey protein. Furthermore, whey protein consumption provides a higher stimulation of protein synthesis after resistance exercise when compared to other protein sources (soy and casein) (Poortmans et al. 2012). Lastly, it has been proposed that whey protein may contribute to immune function (Witard et al. 2014).

Recent studies indicate that the availability of intracellular amino acids, particularly leucine, can represent a key regulator of protein synthesis in skeletal muscle after amino acid intake. Previously, it was believed that the pool of extracellular amino acids was the main responsible for this regulation. More studies are required to clarify this issue, but it appears that both intracellular and extracellular amino acids are important for the regulation of protein synthesis (Dickinson and Rasmussen 2011).

Therefore, the increase in the postprandial situation of MPS seems to be modulated by different factors, such as the source of protein ingested (Baer et al. 2011; Phillips et al. 2009; Reitelseder et al. 2011; Walrand et al. 2016), digestibility, postprandial amino acid availability and hyperaminoacidemia/hyperleucinemia (Mitchell et al. 2015a; Smith et al. 1998, Walrand et al. 2016; West et al. 2011; Paddon-Jones et al. 2006; Tang and Phillips 2009; Tipton et al. 1999; Volek et al. 2013; Volpi et al. 2003).

High protein diets and sarcopenia

The quantity and quality of dietary protein have been shown to impact human health parameters in numerous studies (Wu 2016; Blachier et al. 2010). Among the different metabolic and physiological parameters examined, the impact of alimentary protein on skeletal muscle mass loss has been examined (Murphy et al. 2016). The loss of skeletal muscle mass that starts around the 4th decade of life, named sarcopenia, is a process which occurs at a rate of approximately 0.8% per year, accompanied in some cases by a subsequent increase in adipose tissue. To make a long story short, sarcopenia is a multifactorial and complex process resulting from aging-induced changes in the human organism, including altered muscle fiber type distribution and negative net protein balance (Cruz-Jentoft et al. 2010; Fielding et al. 2011; Gumucio and Mendias 2013; Paddon-Jones and Rasmussen 2009; Riddle et al. 2016; Rosenberg 1997). After the sixth decade of life, the loss of skeletal muscle mass may increase to a rate of approximately 15% per decade. Such muscle loss can be worsened by immobility/disuse/bed rest, changes in endocrine function, chronic diseases, inflammation, insulin resistance and nutritional deficiencies (Coker and Wolfe 2012; Fielding et al. 2011; Malafarina et al. 2013). This progressive loss of muscle mass and strength can impair the quality of life, since activities of daily living become more difficult to be performed (da Silva et al. 2014; Velazquez et al. 2013) and the risk of falls and fractures increase (Landi et al. 2012; Scott et al. 2014).

Furthermore, the skeletal muscle is the largest site of lipid oxidation and consumes a large amount of glucose (Argilés et al. 2016; DeFronzo et al. 1992). Then, losses in this tissue result in lower rates of lipid oxidation and an increase in the blood glucose levels, which may contribute to diabetes type II, obesity, and metabolic syndrome (Bauer et al. 2013; Breen and Phillips 2011; Han et al. 2010; Jurca et al. 2005; Ruiz et al. 2008; Wolfe 2006). The skeletal muscle, concomitantly with the liver, is the most important tissue responsible for thermogenesis (Johnstone et al. 2005). Therefore, skeletal muscle plays a key role in the maintenance of metabolic regulation (Argilés et al. 2016; Wolfe 2006).

As a natural geriatric condition, it is important to seek strategies to counteract this process, given the advancing age of the global population, as predictions suggest that about 200 million people will be affected by sarcopenia in 2050 (Janssen 2011). Sarcopenia can be influenced by lifestyle factors, such as reduced physical activity levels (Nair 2005) and suboptimal protein intake (Volpi et al. 2013). It is well established that consuming protein meals containing EAAs and physical activity—mainly resistance exercise—can stimulate MPS (Burd et al. 2010a, b, 2012; Churchward-Venne et al. 2014a; Volek et al. 2013), therefore, serving as key regulators to maintain muscle mass. However, current evidence suggests that, unfortunately, older adults are less sensitive to the stimulatory effects of the nutrient ingestion (Cuthbertson et al. 2005; Pennings et al. 2012) and physical activity (Durham et al. 2010; Fry et al. 2011; Kumar et al. 2009) on MPS compared with younger population.

A possible cause of this less pronounced MPS after nutrient ingestion by the elderly is due to a decreased capacity of digestion and absorption of protein and amino acids (Boirie et al. 1997), concomitantly with a greater amino acids retention by splanchnic area after intestinal absorption (Boirie et al. 1997; Volpi et al. 1999). This may result in less quantities of amino acids available for MPS stimulation. However, there are discrepancies in studies aiming at determining whether the postabsorptive MPS is reduced with aging. Indeed, although several studies have shown lower levels of MPS in the postabsorptive period in the elderly (Welle et al. 1993; Yarasheski et al. 1993); others have shown no significant difference between young and elderly (Cuthbertson et al. 2005; Katsanos et al. 2006). Two recently published cohort studies (Markofskia et al. 2015; Wall et al. 2015) performed over 10 and 5-year periods, respectively, analyzed the basal MPS of over 300 healthy, non-obese young and older adults and showed no difference in the postabsorptive rates of MPS between young and old individuals. These results give an important information indicating that the basal rates of MPS may not be altered with aging to any significant extent.

However, Markofskia et al. (2015) observed a higher phosphorylation of mTOR and its downstream S6K1 in the elderly despite no changes in MPS. This situation was proposed to be associated with an increased mTORC2 signaling and insulin resistance, together with a deficit in the mTOR phosphorylation after the provision of an anabolic stimuli, such as exercise, insulin and nutrition. In agreement with this hypothesis, the decreased sensitivity of MPS may be related to the lower response to insulin (Rasmussen et al. 2006), which may affect both MPS and MPB. Insulin is released to a higher extent by increased amino acids concentrations (Blachier et al. 1989a, b), although, interestingly, old people appears to be less sensitive to this stimuli

(Biolo et al. 1999; Borsheim et al. 2004; Chow et al. 2006; Fujita et al. 2006; Wilkes et al. 2009). This may lead, therefore, to suppressive effects of hyperinsulinemia in the MPS and MPB (Churchward-Venne et al. 2014b; Fujita et al. 2009). In support of this proposition, a possible cause of the impaired MPS in some elderly, as presented by Wall et al. (2015), could be related to the lower insulin sensitivity; this parameter being reflected by higher glycated hemoglobin, fasting insulin, and Homeostatic Model Assessment of Insulin Resistance index.

Moreover, there is also an apparent lower sensitivity of the aging muscle to the stimulation of MPS by hyperaminoacidemia/hyperleucinemia after protein ingestion (Katsanos et al. 2006; Pennings et al. 2012). This may be due to signaling defects, resulting in reduced phosphorylation of mTORC1 and its substrate p70S6k; as a possible consequence of differences in the expression and function of specific amino acids transporters that can be altered with aging (Cuthbertson et al. 2005; Dickinson et al. 2013; Francaux et al. 2016; Guillet et al. 2004).

Increased oxidative stress (Dardevet et al. 2012) and low grade inflammation (Breen and Phillips 2011; Dardevet et al. 2012; Haran et al. 2012; Ticinesi et al. 2016) may also contribute to reduce the ability of some elderly to display a normal stimulation of MPS. Thereby, all these complex mechanisms may be partly responsible for the reduction of the sensitivity of the aging muscle to the stimulatory effects of protein and amino acids ingestion, leading to an impaired capacity to promote muscle protein synthesis. This condition features the so-called "anabolic resistance" of the senescent skeletal muscle (Burd et al. 2013; Rennie 2009), which is responsible for downregulation of MPS which then may be at lower levels than MPB, resulting in a negative protein net balance. Such a negative net balance may lead, over the time, to a loss of skeletal muscle mass. However, all this factors seems to play a secondary role as a cause of anabolic resistance, since the reduced level of habitual physical activity by the older population is likely to be the main responsible for the aggravation observed in this aging condition (Burd et al. 2013; Breen et al. 2013).

The proposed anabolic resistance observed in some elderly is in relationship with the hypothesis of an "anabolic threshold" or "leucine threshold" regulating the MPS, which has to be reached to initiate the MPS (Dardevet et al. 2012; Norton et al. 2009; Rieu et al. 2006). Therefore, it seems that the older population requires greater concentrations of leucine in the blood circulation to achieve this "threshold" for the same MPS than the young individuals (Bauer et al. 2013; Breen and Phillips 2011; Daly et al. 2014; Dardevet et al. 2012; Katsanos et al. 2006). The study performed by Moore et al. (2009) showed that only ~1 g of leucine (about 10 g of whey protein) is necessary to stimulate MPS above basal levels in young individual muscles at rest, while Yang et al. (2012a) concluded that a minimum amount of ~2 g of leucine (about 20 g of whey protein) are required to rise MPS above basal rates in the elderly at rest.

This issue is of paramount importance as older people tend to consume less dietary protein daily, partly due to reduced energy needs (Fulgoni 2008; Volpi et al. 2013). This remains a serious problem since about one-third of adults who are above the age of 50 consume less than the current recommended dietary allowance (RDA), which is 0.8 g/kgBW/day, whereas approximately 10% of older women do not even reach 0.66 g/kg/BW of protein per day (Houston et al. 2008; Wolfe and Miller 2008; Wolfe et al. 2008). In addition, some elderly ingest small portions of protein in each meal, a fact that contributes to the loss of skeletal muscle mass. Indeed, small quantities of protein seem to have low effects on the stimulation of MPS, even if the quality of protein is high (Katsanos et al. 2005). The situation is further complicated by the fact that older people tend to start the day with small portions of protein (~15 g) and end their day with a meal characterized by high protein content in the evening (~50 g), resulting in a large period of imbalance between MPS and MPB during the day (Berner et al. 2013). This imbalanced protein intake may contribute to decrease the rates of MPS, as the elderly appears to require greater amounts of protein at each meal to raise MPS above basal levels (Moore et al. 2015; Yang et al. 2012a). Then this routine appears to be not ideal for the maintenance of muscle mass with aging, since regular consumption of meals containing protein of high quality ($\geq$0.4 g protein/kg BW) throughout the day may likely be more effective to stimulated MPS (Moore et al. 2015; Murphy et al. 2015; Paddon-Jones and Rasmussen 2009).

This latter hypothesis was recently tested by Mamerow et al. (2014) which performed a 7 days crossover study using isonitrogenous diet to measure the changes in MPS over 24 h with an even or irregular protein distribution in healthy young and middle age adults. The even distribution consisted in approximately 30 g of protein in breakfast, lunch and dinner. Over 24-h period, MPS was 25% higher in the even distribution than in the irregular distribution. The strategy of regular protein consumption appears interesting since the rise of MPS by protein intake is transient, reaching the peak within approximately 2 h and returning to basal levels in about 3–4 h after ingestion (Atherton and Smith 2012; Bohe et al. 2001; Dickinson and Rasmussen 2011; Phillips 2014). Furthermore, old people seem to have higher protein needs than young individuals, with values around 1.2–1.6 g protein/kg BW/day (Bauer et al. 2013; Calvani et al. 2013; Wolfe and Miller 2008, 2008). However, the data obtained in this area are rather heterogeneous. Indeed, in a recent publication by Kim et al. (2015), no effect of the protein distribution pattern on the MPS

responses was recorded during an entire day. These findings contrast with the results of Mamerow et al. (2014), maybe because the population studied was different (elderly vs. young/middle age adults) in the two studies. An ingestion of 0.4 g/kg/BW of high-quality protein appears to be the minimum amount required to stimulate muscle anabolic response (Moore et al. 2015).

When ingested in the context of a mixed meal, it is possible that a greater amount of high-quality protein is required to achieve the maximal MPS, as this condition is associated with less amino acids bioavailability (Burke et al. 2012). Nevertheless, it still can be recommended to fractionate the protein intake during the day, since older people frequently feature a decreased level of appetite (Landi et al. 2016), a condition that may hamper the consumption of a meal with very high protein content, as seem in the study by Kim et al. (2015). It is worth noting that the main difference between the higher vs. lower protein ingestion groups was the increased rates of MPS in the higher protein ingestion group, a result in accordance with other findings (Bauer et al. 2013; Calvani et al. 2013; Wolfe and Miller 2008, 2008) and which suggests that older individual may benefit from higher consumption of protein during the all day.

A recent work (Loenneke et al. 2016) found a positive association between protein quantity per meal and leg lean mass and strength. In fact, consuming frequent meals containing 30–45 g of protein is associated with greater leg lean mass and knee extensor muscle strength. Such nutritional strategy was also associated with higher LBM and appendicular LBM in both older men and women at baseline and after a 2-years follow-up period (Farsijani et al. 2016). These findings reinforce those provided by Loenneke et al. (2016). However, further long-term longitudinal research is required to determine an optimal protein intake quantity and distribution throughout the day to help to preserve skeletal muscle mass and function in older individuals.

The difficulty to reach the optimal value of protein intake during the day by the elderly may lead to a protein deficit, which chronically may induce skeletal muscle wasting. To counteract this situation, high protein diets combined with high-quality protein supplement may help for the preservation of skeletal muscle mass (Cermak et al. 2012; Deer and Volpi 2015; Malafarina et al. 2013; Wolfe 2012). To support this proposition, some studies (Geirsdottir et al. 2013; Houston et al. 2008) have found a positive correlation between consumption of protein and maintenance of skeletal muscle mass in older adults, since higher amounts of protein intake was correlated with an increased skeletal muscle mass retention over the time; suggesting a possible protective effect of protein against muscle wasting when amounts of protein near or even above the recommended values are ingested.

Consumption of whey protein instead of protein meals has been proposed as a possible strategy to reach the daily requirements of protein able to stimulate MPS in elderly. A study performed by Pennings et al. (2012) compared the rates of protein accretion after the ingestion of three different doses of whey protein (10, 20, 35 g) and showed positive results for the 20 and 35 g-consuming groups while the MPS displayed no significant difference after the ingestion of the 10 g protein dose. This result is in agreement with the findings from Wall et al. (2015) which suggest that higher doses of protein with high biological value at rest are necessary to optimally stimulate MPS in older men. It may explain why Arnal et al. (1999) and Bouillanne et al. (2013) did not find positive results under their experimental design. Indeed, Bouillanne et al. (2013) fail to demonstrate any benefit from 6 weeks dietary intervention in hospitalized older adults, with most meals containing less than 20 g protein. Similar results were obtained in the study by Arnal et al. (1999). Thus, it appears that 20 g protein is the minimal amount required to promote MPS in elderly at rest (Yang et al. 2012a).

Positive results have been obtained by Tieland et al. (2012b) after the intake of the supplement in breakfast and lunch of frail elderly. In this latter study, protein intake increased to more than 25 g of protein in each main meal ($\geq$0.34 g/kg/BW per meal), allowing improvements of both strength and physical performance despite no increase in skeletal muscle mass after 24 weeks.

In a similar manner, an optimized offer of high-quality protein in the breakfast and lunch (all daily meals containing $\geq$0.4 g/kg/BW) by protein supplementation promotes a significant increase in appendicular lean tissue mass in healthy older individuals (Norton et al. 2016). In this latter and other studies, the average compliance to the treatment was very high ($\geq$92%) (Norton et al. 2016; Tieland et al. 2012b). In addition, since the protein-induced suppression of energy intake may be blunted in the elderly (Giezenaar et al. 2015; Norton et al. 2016), this may explain why the maintenance of energy balance and consequently of a positive skeletal muscle protein balance were observed (Carbone et al. 2012; Hector et al. 2015; Pasiakos et al. 2015).

Physical activity may also play an import role for the maintenance of skeletal muscle mass and function. Resistance training combined with milk protein supplementation given in the breakfast and lunch of frail elderly gave promising results since this combined stimuli increases by 1.3 kg lean body mass with no differences in the placebo group (Tieland et al. (2012a). However, similar to others findings (Norton et al. 2016; Tieland et al. 2012b), and as pointed out above, there was an increase in the total daily protein intake from 1.0 g/kg/BW up to 1.3 g/kg/BW, rendering impossible to attribute these results only to the protein distribution pattern.

Yang and colleagues (2012a), by combining resistance exercise in older men with post-exercise whey protein supplementation, found that different doses of protein, i.e., 10 g (0.13 g/kg BW), 20 g (0.25 g/kg BW) and 40 g (0.49 g/kg BW) gave positive results. They found that, as observed by Pennings et al. (2012) who demonstrated better results in MPS with higher doses of protein ingestion at rest; 40 g of whey protein intake given after resistance training was able to stimulate myofibrillar protein synthesis by 91% above the placebo group; while the group that consumes 20 g of whey displayed only 44% increase above the value obtained in volunteers without supplementation. In agreement, D'Souza et al. (2014) found a linear relationship between whey protein dose and muscle p70S6k phosphorylation, wherein 40 g of protein intake results in greater levels of p70S6k phosphorylation than lower protein doses. Phosphorylation of p70S6k is often correlated to increases in MPS rates and possible muscle hypertrophy (Baar and Esser 1999; Mitchell et al. 2013; Terzis et al. 2008). Although, other mechanisms are likely to be also involved in the skeletal muscle hypertrophy. In fact, in some cases there is a lack of correlation between acute changes in phosphorylation of p70S6k and long-term skeletal muscle hypertrophy (Mitchell et al. 2012, 2014). These findings provide an important information suggesting that older people have higher need for high-quality protein to optimize the stimuli of muscle protein synthesis at rest and after resistance exercise.

In agreement with these results, a recently published randomized, double-blind, placebo-controlled supplementation trial (Rondanelli et al. 2016) that combined whey protein, essential amino acids, and vitamin D with regular physical activity in 130 sarcopenic elderly people (53 men and 77 women; mean age 80.3 years) showed positive results of the dietary intervention. After 12 weeks of intervention with physical activity 5 times/week and ingestion of 32 g of a supplement containing 22 g of whey protein (~4 g of leucine) and 2.5 g of vitamin D one time per day, 68% of the sarcopenic elderly became non-sarcopenic with a gain of ~1.7 kg in fat free mass. These results were concomitant with others improvements in health parameters, such as reduced inflammatory state, increased performance on activities of daily living, and enhanced IGF-1 concentrations. These results suggest that physical activity is important, but alone is not sufficient, to achieve significant results since placebo group did not present all these improvements. However, the physical activity was non intensive in this latter study, suggesting that it could have been a limiting factor for the increase in fat free mass. Furthermore, it is important to notice that despite the relatively low dose of whey protein used (22 g), the high content of these protein in leucine and the vitamin D supplementation might explain the positive and ample results of the study.

Current evidence suggests that performing exercise before protein intake allows greater use of dietary protein-derived amino acids for de novo muscle protein synthesis in elderly men (Pennings et al. 2011b). Thus, based on these data, physical activity positively impacts the ability of skeletal muscle to retain dietary amino acids, in both young and old individuals, and improves the anabolic response of a meal containing protein (Devries et al. 2015; Timmerman et al. 2012; Walker et al. 2011). A bout of moderate intensity aerobic exercise seems to be efficient to sensitize the skeletal muscle and enhances the anabolic effects of protein feeding (Timmerman et al. 2012). Although the enhanced sensitivity of MPS after an intense bout of resistance exercise persists up to 24 h (Damas et al. 2015), the greatest exercise-mediated increases in MPS occurs immediately after the exercise bout (Churchward-Venne et al. 2012). Thus, providing frequent daily stimulus such as walking to enhance MPS may be valuable for the elderly to maintain the maximal skeletal muscle response to protein-induced increases in MPS during the day.

Due to anabolic resistance, as defined above, an important issue that thus appears determinant for the stimulation of skeletal muscle protein synthesis is the protein source. Indeed, there are different types of dietary proteins with different characteristics, such as the digestibility, amino acids composition, and rates and kinetics of absorption of these AAs. Such parameters would likely lead to distinct MPS responses. Data provided by Yang et al. (2012b) compared the MPS responses after intake of different protein sources in the elderly. These authors assessed the effects of whey protein and soy protein ingestion under rest and post-exercise conditions. They compared three different doses of protein supplementation, that are 0, 20 and 40 g of whey protein or soy protein on the rates of leucine oxidation and fractional synthesis rates (FSR). The results pointed to higher increases in FSR in both rested and after resistance exercise using whey in comparison with soy protein, despite lower rates of leucine oxidation when small doses of protein were ingested. Similar results were found by Mitchell et al. (2015a), which showed a less prolonged p70S6k phosphorylation after soy protein ingestion (~2 h) compared to whey protein (~4 h) after a session of resistance exercise.

Whey protein also seems to be more effective to increase the rates of MPS when compared with casein. A study performed by Burd et al. (2012) revealed that the ingestion of whey protein elicited greater rates of MPS against its casein counterpart in older adults. It is important to notice that this latter study also tested the subjects at rest and after resistance training, and found, in both conditions, whey to give better results than casein. This result is probably related to a greater hyperaminoacidemia or hyperleucinemia after whey ingestion, due to the kinetic of digestion

of each protein. Findings from Pennings et al. (2011a, b) is in accordance with these results, as greater circulating concentrations of leucine were measured after whey ingestion compared with casein ingestion. Moreover, high leucine plasma concentrations combined with peak leucinemia resulting from whey supplementation displayed a strong relationship with MPS (Pennings et al. 2011a, b). Walrand et al. (2016) recently compared in the elderly the rates of synthesis in individual muscle protein fraction—sarcoplasmic, mitochondrial, actin and myosin—after the intake of casein and a fast-digestive milk protein with different amounts of leucine. The consumption of both 15 and 30 g of the fast-digestive milk protein was able to increase mitochondrial protein FSR, while no difference was elicited in the postprandial period after casein ingestion, suggesting that each protein source has an unique effect at specific muscle protein fractions.

However, although myosin protein FSR was stimulated after both protein sources, myosin FSR was enhanced with 30 g but not with 15 g of casein. Sarcoplasmic muscle protein and actin FSRs were not significantly increased in the postprandial state compared to the postabsorptive state. It is worth to note that the availability of plasma leucine was higher for both 15 and 30 g of fast-digestive milk protein, despite 30 g of casein contain larger amounts of leucine than 15 g of rapidly digested milk protein.

These results point to a better response of MPS after whey ingestion despite the fact that casein (only when hydrolyzed) and soy protein also display fast digestibility, high bioavailability, and high EAAs content. Whey protein consumption results in a higher concentrations of leucine and promotes an overall rapid increase of amino acid concentrations in plasma. Such increase apparently is the "trigger" to start the cell signaling process allowing protein synthesis (Katsanos et al. 2006; Layman et al. 2015; Volpi et al. 2003; West et al. 2011). Concomitantly, the role of each source of protein in the human metabolism may contribute to the differences in the MPS stimulation after the ingestion of a given mixture of nutrients. Indeed, for instance, amino acid derived from whey protein in milk or from soy protein can be used differently both in the splanchnic and peripheral tissues. Amino acids derived from soy protein seems to support greater splanchnic protein synthesis and to be converted to urea to a greater extent than milk proteins. This may be due to the high content of BCAAs in whey protein, since it contributes to less splanchnic extraction (Bos et al. 2003; Fouillet et al. 2002; Luiking et al. 2005). These different metabolic fates may explain, in part at least, the superiority of milk protein to rise MPS compared to soy protein (Hartman et al. 2007; Wilkinson et al. 2007).

Without consideration regarding the different dietary sources, high quantities of protein may be efficient to stimulate MPS above basal rates when combined with resistance training in elderly. Some studies have demonstrated that 40 g of beef meat, whey and soy protein, were able to raise MPS around 80–100% after resistance training. However, low doses (~10 g) were unable to increase the protein accretion (Robinson et al. 2013; Yang et al. 2012a, b).

However, in contrast to the findings above, two recently published data in both young individuals (Mitchell et al. 2015b) and in the elderly (Mitchell et al. 2015c) suggest that a sustained EAA delivery profile (pulse feeding) may be as good as a rapid rise of aminoacidemia (bolus feeding) to stimulate MPS at rest. More studies applying this latter strategy are needed to explore potential clinical benefits of such a strategy for the elderly.

Taken together, these findings show that most elderly people do not reach the necessary amounts of protein to counteract the anabolic resistance and to attenuate the subsequent sarcopenia. A strategy that seems to be useful to help old people to ingest their protein daily requirements is through protein supplementation, mainly in the form of whey protein due to their relatively high EAAs content (notably with high amounts of leucine), fast and high digestibility and good bioavailability. All these characteristics are propitious to promote a rapid and robust rise in leucinemia, likely allowing high rates of MPS. Thus, providing three or more high-quality, leucine-rich protein meals during the day combined with regular physical activity may represent a good strategy to attenuate aging-related skeletal muscle mass losses (Fig. 1).

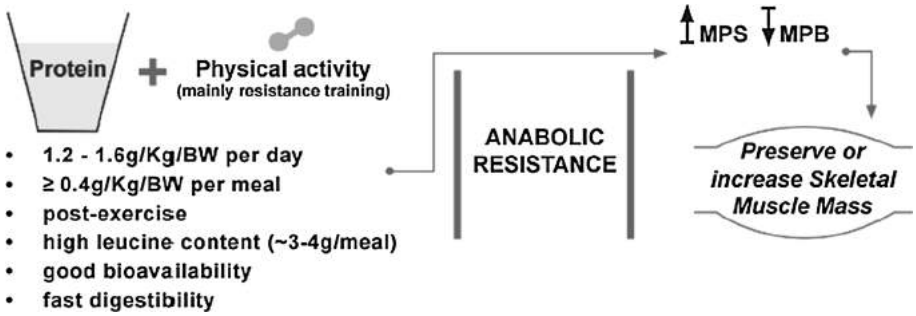

Fig. 1 Schematic view showing how the key variables of protein ingestion and physical activity can influence the skeletal muscle mass in the elderly. *MPS* muscle protein synthesis, *MPB* muscle protein breakdown

High protein diet and renal impairment

The high protein intake leads to oxidation of the amino acids in excess and possible metabolism in triglycerides and glucose. Excessive protein intake results in an elevated nitrogen rates in the body, which leads to a greater glomerular filtration rate, and increases the production of urea (Marckmann et al. 2015; Poortmans et al. 2012). There was no effect of a high protein intake on renal function, such as glomerular filtration, excretion of albumin and calcium metabolism in trained athletes consuming less than 2.8 g/kgBW per day of protein (Poortmans and Dellalieux 2000). In another study, the renal function was examined by the measurement of the glomerular filtration rate, after 12 weeks of intervention with resistance training and protein supplementation in elderly. The results showed no impairment of glomerular filtration rate with a protein consumption of approximately 1.0 g/kgBW (Ramel et al. 2013).

The current recommendation suggests a moderate/restricted protein consumption, mainly in the elderly, only in the presence of a moderate/severe kidney disease with no dialysis (Bauer et al. 2013).

High-protein diet and colon health

An increase in the amount of dietary protein intake increases the quantity of unabsorbed proteins, peptides and amino acids reaching the large intestine through the ileocecal junction (Gibson et al. 1976). In the small and large intestines, bacteria in the luminal contents can metabolize proteins and amino acids (Dai et al. 2012; Blachier et al. 2010; Andriamihaja et al. 2013); and metabolomics analysis has revealed different profiles in the plasma and urine of rodents receiving a high-protein diet as compared to diet containing a moderate amount of proteins (Mu et al. 2015). In the large intestine, undigested and partially digested dietary protein and peptides from dietary and endogenous origin are degraded by endogenous and bacterial proteases/peptidases in peptides and amino acids; these latter being not absorbed by the colonic epithelium but serving as precursors for numerous bacterial metabolites including ammonia, hydrogen sulfide, amines, short-chain and branched-chain fatty acids, indoles, phenols, ethanol and organic acids, etc. (Liu et al. 2014; Mouillé et al. 2004; Davila et al. 2013). Several among these metabolites (H_2S, NH_4^+, *p*-cresol) have been shown at excessive concentrations to affect colonic epithelial energy metabolism (Beamount et al. 2016; Andriamihaja et al. 2010, 2015). *p*-Cresol which is produced by the microbiota from L-tyrosine shows genotoxic effect on colonocytes (Andriamihaja et al. 2015). In addition, hydrogen sulfide has been recently shown to drive mucin denaturation, and to presumably reduce mucus barrier function in the colon (Ijssennagger et al. 2015). High-protein diet given for 2 weeks has been shown to modify colonic epithelial cell morphology (Andriamihaja et al. 2010) and to affect the distribution of mucous cells in the colonic epithelium (Lan et al. 2015), maybe due to alteration in the process of colonic epithelial cell proliferation and differentiation. These results were all obtained in animal models. In human studies, it has been shown that high protein weight loss diet promotes in the colonic lumen a bacterial metabolite profile that is likely detrimental for colonic health (Russell et al. 2011); suggesting that long-term adherence to such diet may increase risk of colonic diseases. Since there is no upper limit for protein intake with no adverse effects, some caution is advised regarding long-term consumption of high protein diet particularly regarding the maintenance of the colonic health.

Conclusions

From the available data, it appears necessary in the elderly population to examine each case individually to determine the actual relevance for protein dietary supplementation. Furthermore, one must take into account the origin of the substances that will be used for supplementation, as well as the safety for use and evidence of its effectiveness.

A key point regarding the effectiveness of protein supplementation is the combination of training, food intake and supplementation. Considering that muscle hypertrophy is only due to protein supplementation has simply no scientific support. Several studies showed some positive results on the association of physical exercise together with supplementation of whey protein, especially for audiences like the elderly who may experience protein deficit. It is worth to note that protein sources with high biological value containing essential amino acids (notably leucine), such as whey protein, but of course not exclusively, are most relevant to generate the appropriate stimulus for protein synthesis. The Table 1 presents a list of studies related to the effects of dietary protein supplementation in the elderly.

Regarding the recommended dose, an adequate protein intake from 1.2 up to 1.6 g/kg/BW during the day may be required by the elderly to preserve muscle mass. Fractionate protein intake in daily doses may likely be a good strategy since older individuals may experience diminished appetite. Therefore, an individualized nutritional strategy should always be designed to successfully reach the recommended daily protein needs. However, since the upper non deleterious level of dietary protein remains undefined, and taking into consideration that high protein diet in long-term utilization may impact colon health, some additional studies are required to test in healthy volunteers the impact of high-protein diet on the large intestine epithelium. In that

Table 1 Summary of the quoted studies related to the effects of dietary protein supplementation in aging individuals and in the elderly

Study	Duration	Subjects	Protocol	Outcome
Burd et al. (2012)	Acute	14 healthy aging men	Ingestion of 20 g of casein or whey at rest and after an acute bout of RE	Greater myofibrillar MPS after whey than casein in both conditions
Pennings et al. (2012)	Acute	33 healthy aging men	Ingestion of 10, 20 and 35 g of whey protein at rest	Muscle protein accretion 35 g > 20 g > 10 g
Wall et al. (2015)	Acute, retrospective, cross sectional study	35 healthy young men; 40 healthy elderly men	Ingestion of 20 g of casein at rest	16% lower post-prandial FSR in elderly than in young men
Francaux et al. (2016)	Acute	9 healthy young men; 10 healthy aging men	Ingestion of 30 g of whey protein after an unilateral RE	↑ PKB phosphorylation in young than elderly in both rested and after RE ↑ mTOR in both age groups after RE ↓ S6K1 in all conditions in the aging men
Yang et al. (2012b)	Acute	30 healthy elderly men	Ingestion of 0, 20, 40 g of whey protein (WP) or soy protein (SP) at rest and after a bout of RE	Leucine oxidation: SP20 > WP20; SP40 = WP40 MPS at rest: SP0 = SP20 = SP40; WP40 > WP20 > WP0 MPS after RE: SP40 > SP0 = SP20; WP40 > SP40 = WP20
Moore et al. (2015)	Acute	65 healthy young men; 43 healthy aging men	Ingestion of varying amounts (0–40 g) of high-quality dietary protein as a single bolus and normalized to body mass	No differences in basal MPS between the groups MPS reached a plateau after ingestion of 0.40 g/kg/BW and 0.24 g/kg/BW in aging and young men, respectively
D'Souza et al. (2014)	Acute	46 healthy elderly untrained men	Ingestion of 10, 20, 30 or 40 g of whey protein or placebo after a bout of RE	↓ Intramuscular BCAAs levels after RE in placebo group ↑ Intramuscular BCAAs levels after 2 h post RE in the 30 and 40 g whey protein groups, and after 4 h only in 40 g whey protein group Significantly increases in p70S6K phosphorylation up to 4 h only after ingestion of ≥20 g of whey protein. Best results after 30 and 40 g of whey protein
Mitchell et al. (2015c)	Acute	33 healthy aging untrained men	Ingestion of 30 g of whey protein, soy protein, CHO or placebo	Increased p70S6K in whey and soy protein group after 2 h post RE After 4 h post RE p70S6K remained elevated only in whey protein group
Pennings et al. (2011a)	Acute	48 aging men	Ingestion of 20 g whey, casein or casein hydrolysate	FSR values: whey > casein hydrolysate > casein
Pennings et al. (2011b)	Acute	24 young and 24 aging men	Ingestion of 20 g casein at rest or after a 30 min exercise protocol	Greater use of AAs for MPS after exercise in young and elderly man
Farnfield et al. (2012)	Acute and chronic (12 weeks)	16 healthy young men; 15 healthy aging men	Ingestion of 27 g of whey protein or placebo after an acute bout of RE pre and post 12 week RE program	Similar increases in strength in both groups for each category Pre RE: ↑ mTOR, 4E-BP1 after whey than placebo in both ages ↑↑ p70S6K after whey in older compared with placebo ↑ eIF4G after whey than placebo in older Post RE: slightly blunted mTOR pathway phosphorylation after whey ingestion in aging men

Table 1 continued

Study	Duration	Subjects	Protocol	Outcome
Walrand et al. (2016)	Chronic, 10 days	31 healthy elderly men	Ingestion of 15 g adequate protein (AP) or 30 g high-protein (HP) diets of Casein (CAS) or soluble Milk protein (PRO)	↑ Myosin FSR after both HP groups, but only in PRO AP ↑ Mitochondrial FSR in both PRO quantities
Norton et al. (2016)	Chronic, 24 weeks	60 healthy ageing men and women	Ingestion of 0.165 g/kg body mass of a milk-based protein matrix (PRO) or an isoenergetic, non-nitrogenous maltodextrin control (CON) at breakfast and midday meals	Increased daily protein ingestion from 1.2 to 1.6 g/kg/BW in the PRO group Protein intakes in the PRO group increased to ≥0.4 g/kg per meal LTM increased by 0.45 kg in PRO group and decreased 0.16 kg in CON group
Tieland et al. (2012b)	Chronic, 24 weeks	65 pre-frail and frail elderly men and women	Ingestion of 15 g of milk protein (PRO) or placebo after breakfast and lunch	Increased daily protein ingestion from 1 to 1.4 g/kg/BW in the PRO group Protein intake in the PRO group increased to more than 25 g per meal Improved physical performance in PRO group compared with placebo group
Tieland et al. (2012a)	Chronic, 24 weeks	62 frail elderly subjects	Ingestion of 15 g of milk protein (PRO) or placebo after breakfast and lunch Participation in a progressive resistance-type exercise training program 2× per week	Increased daily protein ingestion from 1 g/kg/BW to 1.3 g/kg/BW in the PRO group ↑ LBM in protein group, no changes in placebo group Strength and physical performance improved significantly in both groups
Rondanelli et al. (2016)	Chronic, 12 weeks	130 sarcopenic elderly (53 men; 77 women)	Ingestion of nutritional supplement (22 g whey protein, 4 g leucine, 2.5 g vitamin D) or placebo Both groups participated in a five times/week physical activity program	Fat free mass increased 1.7 kg in the supplemented group; no changes observed in placebo group Significant improvements in relative skeletal muscle mass, strength, inflammatory state and physical function in the supplemented group; no changes observed in placebo group 68% of elderly in the supplemented group became non-sarcopenic

RE resistance exercise, *MPS* muscle protein synthesis, *FSR* fractional synthesis rate, *PKB* protein kinase B, *mTOR* mammalian target of rapamycin, *p70S6K1 or S6K1* ribosomal protein S6 kinase 1, *BCAAs* branched-chain amino acids, *AAs* amino acids, *4E-BP1* eukaryotic translation initiation factor 4E-binding protein 1, *eIF4G* eukaryotic translation initiation factor 4G, *LTM* lean tissue mass, *LBM* lean body mass, *FFM* fat free mass, *RSMM* relative skeletal muscle mass

regard, supplementation with individual amino acids may represent on some occasions an interesting strategy, taking into account the large capacity of the small intestine for their absorption.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict ofinterest.

Ethics statement The manuscript has not been submitted to other journal for simultaneous consideration. The manuscript, partly or in full, has not been published previously. No data have been fabricated or manipulated (including images) to support our conclusions. No data, text, or theories by others are presented as if they were the author's own (plagiarism).

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