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Dietary protein considerations for muscle protein synthesis and muscle mass preservation in older adults

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Abstract

Amino acid bioavailability is critical for muscle protein synthesis (MPS) and preservation of skeletal muscle mass (SMM). Ageing is associated with reduced responsiveness of MPS to essential amino acids (EAA). Further, the older adult population experiences anabolic resistance, leading to increased frailty, functional decline and depleted muscle mass preservation, which facilitates the need for increased protein intake to increase their SMM. This review focuses on the role of proteins in muscle mass preservation and examines the contribution of EAA and protein intake patterns to MPS. Leucine is the most widely studied amino acid for its role as a potent stimulator of MPS, though due to inadequate data little is yet known about the role of other EAA. Reaching a conclusion on the best pattern of protein intake has proven difficult due to conflicting studies. A mixture of animal and plant proteins can contribute to increased MPS and potentially attenuate muscle wasting conditions; however, there is limited research on the biological impact of protein blends in older adults. While there is some evidence to suggest that liquid protein foods with higher than the RDA of protein may be the best strategy for achieving high MPS rates in older adults, clinical trials are warranted to confirm an association between food form and SMM preservation. Further research is warranted before adequate recommendations and strategies for optimising SMM in the elderly population can be proposed.

Key words: **Muscle protein synthesis: Elderly: Essential amino acids: Ageing: Dietary protein**

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Introduction

The older adult population is rapidly growing with over 600 million people globally being aged 65 years or older[1]. This increased ageing population is associated with an increase in the prevalence of chronic diseases such as CVD, hypertension, obesity and diabetes[2]. CVD affects over 40 million adults above the age of 65 years in the USA[3] and remains the number one cause of death globally[4]. The global prevalence of diabetes is increasing, particularly in older adults, and the number of cases is expected to double in the next two decades[5]. Currently, over 25 % of older adults in America are diabetic and 51 % are pre-diabetic[5]. In Europe, the prevalence of diabetes in older adults is on average 20 %, with variations among nations ranging from 14–16 % in Denmark to 31 % in Greece[5]. A recent European study, involving over twenty countries, reported a significantly higher incidence of overweight and obesity in older adult populations, compared with middle-age and younger adults[6]. On a global level, more than 650 million adults were reported as obese in 2016[7]. Furthermore, a European countries report estimated that 20 million people aged >50 years have osteoporosis[8]. These data confirm that these diseases are a serious worldwide public health concern and are prevalent in the older adult population. Thus, in this growing population identifying ways to promote healthy ageing, through diet and exercise, has never been more important.

Skeletal muscle mass (SMM) is largely associated with physical function and athletic performance. Preserving SMM is essential for maintaining metabolic health and can play an essential role in minimising the risk of diseases, such as CVD, obesity, diabetes and osteoporosis[9]. SMM is determined by a fine balance between muscle protein synthesis (MPS) and muscle protein breakdown. Though the exact mechanisms are unknown, essential amino acids (EAA) play a role in MPS by functioning as signalling molecules to induce MPS[10]. To date, studies have primarily focused on leucine, which is recognised as the most potent amino acid (AA) to stimulate a postprandial MPS response[11,12]. MPS is also influenced by the myostatin–Smad2/3 pathway, a major signalling pathway which acts as a negative regulator of protein synthesis[13]. It is important to acknowledge that MPS is not the only controlling factor in muscle mass accretion/loss. Transcription factors like SRF (serum response factor) help regulate skeletal muscle growth[13] and androgens and b2-adrenergic agents can have anabolic effects on skeletal muscle[14,15]. In addition, strength and conditioning exercises can positively increase SMM.

The availability of protein, more specifically AA, is important for MPS[16]; thus dietary protein is a key stimulus for preserving SMM. In addition, AA availability is influenced by protein source, digestibility, absorption kinetics and protein intake pattern[17,18]. The international RDA for protein is 0·8 g/kg body weight per d,

Abbreviations: EAA, essential amino acid; MPS, muscle protein synthesis; mTOR, mammalian target of rapamycin; mTORC, mTOR complex; PPF, protein pulse-feeding; SMM, skeletal muscle mass.

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irrespective of age[19]. However, the International PROT-AGE Study Group[20] and the European Society for Clinical Nutrition and Metabolism (ESPEN) recommend that the daily protein requirement for healthy individuals over 65 years is 1·0–1·2 g protein/kg body weight[21]. Furthermore, several studies advise that consuming higher than the RDA of protein may help preserve muscle mass[17,20-22]. Ageing can be associated with reduced food intake[23] and anabolic resistance (losing the ability to build muscle mass) is exacerbated with age[16]. Thus, strategies are needed to help increase protein intake and preserve SMM in older adults. One strategy would be to consider the specific nutritional requirements and physiology of older adults and design functional foods that are a good source of dietary protein that when consumed have a positive effect on MPS and SMM.

The broad nature of muscle hypertrophy and protein metabolism means that its complexity cannot be discussed in one review. The primary focus of this review is to examine the role of dietary protein on muscle mass preservation during ageing, by exploring the role of EAA and protein intake patterns. In addition, to consider novel dietary strategies for optimising SMM preservation in older adults, factors such as protein source, food form and digestibility are also discussed. Finally, the potential use of protein blends in the design of functional foods and the benefits of 'snack' functional foods for older adults will be considered.

Essential amino acids and muscle protein synthesis

Maintenance of muscle mass is achieved through a balance between MPS and muscle protein breakdown rates. To gain SMM, MPS rate must exceed the rate of muscle protein breakdown and when the converse happens skeletal muscle wasting can occur[24]. The availability of AA, in particular EAA, is important in the stimulation of MPS[25]. EAA activate the mammalian target of rapamycin (mTOR) signalling pathway in muscle cells, which serves to integrate intracellular and extracellular signals that regulate cell growth and metabolism[26]. Increased plasma aminoacidaemia means more AA are available to stimulate mTOR for increased MPS[22], and therefore preservation of muscle mass. The exact mechanism by which EAA induce the mTOR pathway (Fig. 1) has been explored in several studies[27–32]. The mTOR protein is a 289-kDa serine-threonine kinase that exists in two complexes, mTOR complex 1 (mTORC1) and mTORC2, which are important modulators of cell growth and proliferation[27,28]. Findings from a recent study in cultured SH-SY5Y cells and mouse embryonic fibroblasts indicate that tyrosine kinase Src plays a crucial role in the AA mediation of mTORC1[29]. Src regulates mTORC1 activity via Rag GTPases[30], by causing the separation of Gap Activity TOward Rags 1 (GATOR1), a Rag GTPase-activating protein, from the Rag proteins[29]. The Rag GTPases are composed of RagA or RagB bound to RagC or RagD[29]. In addition, Sestrin2 has been identified as one of the proteins involved in sensing and signalling AA, in particular leucine, to the Rag GTPases[31].

The role of leucine

Leucine plays an important role in stimulating MPS, with several *in vivo* studies reporting its effects on MPS in both animal[12] and human[11] skeletal muscle. The mechanism by which leucine improves muscle mass is by acting as a potent activator of the mTOR pathway[33]. Several studies have been conducted in older adults investigating the impact of leucine on MPS and its role in muscle mass preservation in this cohort (Table 1). Katsanos *et al.*[34] reported that increasing dietary leucine from 1·7 g to 2·8 g caused a comparable increase in postprandial MPS rates in elderly participants (average age 66·6 years) compared with young adult participants (average age 29·7 years). The beneficial effects of leucine were further demonstrated in a study involving elderly men, where ingestion of leucine (2·5 g) with pure dietary protein (casein) increased postprandial MPS rates[35]. Even in the case of hyperaminoacidaemia, the addition of leucine to complex meals can efficiently increase MPS, as observed in a study where supplementation of mixed meals with 0·052 g leucine/kg body weight significantly increased MPS rates in healthy elderly men[36]. Furthermore, co-ingestion of leucine (5 g) three times per d (with each meal) can increase MPS under resting conditions, irrespective of total protein intake[37], suggesting that leucine makes an impact on MPS in older adults without the need to alter food intake. The authors proposed, however, that the increase in MPS over 3–5 d may only have been an initial response to additional leucine and may not have been sustained over a longer period of time[37]. Therefore, future studies should have a longer duration to determine if MPS is sustained. Overall, these studies suggest that the addition of free leucine to meals represents an effective strategy to augment postprandial MPS in older adults.

Current data suggest that giving doses above 7 g EAA, containing at least 2·5 g leucine per meal, is sufficient to increase MPS rates, with potential to attenuate muscle wasting in older adults (Table 1). However, it is important to note that in these studies muscle atrophy was not measured following leucine supplementation; therefore it remains inconclusive as to whether leucine supplementation can benefit muscle wasting conditions. As well as improving postprandial MPS, increasing leucine in the diet can improve muscle mass and strength in the elderly. Dillon *et al.*[38] reported increases in lean muscle mass (3·9 %) in elderly women following consumption, twice daily for 12 weeks, of a leucine-rich EAA supplement containing 1·39 g leucine (Table 1). In an additional study, comprised of a similar age group, a leucine-rich EAA supplement containing 3·95 g leucine, taken twice daily for 16 weeks (Table 1), resulted in a significant ($P<0·05$) increase in lean body mass and muscle strength even in the absence of exercise[39]. However, contrary to this, some studies report that leucine supplementation does not have an impact on MPS and muscle mass preservation, even when total protein intake was greater than the RDA[40,41]. Verhoeven *et al.*[42] and Leenders *et al.*[43] reported that chronic leucine supplementation caused no changes to SMM or strength in healthy and diabetic elderly men. One review article hypothesised that leucine supplementation had no effect on muscle mass when the leucine signal and the rise in AA no longer function in sync. This can occur due to rapid absorption of free leucine following digestion, while other AA are released at a later stage after digestion[44]. The delayed release of AA means that protein synthesis was only stimulated for a short period postprandially;

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Table 1. Studies investigating the impact of various essential amino acids on muscle protein synthesis (MPS) and muscle protein breakdown in the elderly

Study	Subjects	Treatment	Treatment duration	Key findings
Børsheim *et al.* (2008)[39]	*n* 12, age > 67 years	Test: 11·0 g EAA (containing 3·95 g Leu) twice per d + Arg (2·2 g) once per d between meals	4 months	↑ LBM (1·3 %) ↑ Leg strength (22 %) ↑ Physical function
Dillon *et al.* (2009)[38]	*n* 14, age > 68 years	Test: 7·5 g EAA twice per d (containing 2·78 g Leu) Placebo: lactose, equal to the EAA supplement in weight and energy content	3 months	↑ LBM (3·9 %)
Ferrando *et al.* (2010)[45]	*n* 21, age > 65 years	Test: 15·0 g EAA three times per d (containing 5·38 g Leu) Placebo: non-energy diet soda	10 d	May preserve muscle function but no reduction in loss of lean mass
Katsanos *et al.* (2005)[46]	*n* 11, age > 68 years *n* 8, age 31–68 years	Test: 6·7 g EAA (containing 1·7 g Leu) once per d	1 d	Elderly = no change in MPS rates Young = ↑ MPS rates
Katsanos *et al.* (2006)[34]	*n* 2, age > 66 years *n* 2, age > 28 years	Test: 6·7 g EAA containing Leu (1·7 g) or Leu (2·8 g) once per d	1 d	2·8 g Leu = comparable MPS rates with the young
Leenders *et al.* (2011)[43]	*n* 60, age > 71 years	Test: 2·5 g Leu three times per d with standardised meal Placebo: 2·5 g wheat flour three times per d with standardised meal	6 months	No change in SMM or strength
Murphy *et al.* (2016)[37]	*n* 20, age > 65 years	Test: 5·0 g Leu three times per d with meal Placebo: 5 g alanine and glycine three times per d with meal	3–5 d	Leu co-ingestion = ↑ MPS rates
Rieu *et al.* (2003)[41]	*n* 20, age > 69 years	Test: 0·052 g Leu/kg body weight, 0·4 g casein, carbohydrate, valine, isoleucine, oil, glycerol monostearate, raspberry aroma once per d Control: same as test but no Leu, valine or isoleucine, with added alanine (0·071 g/kg)	1 d	Significant ↑ in MPS rates
Symons *et al.* (2007)[47]	*n* 10, age > 41 years *n* 10, age >70 years	Test: 113·0 g lean beef containing 10·0 g EAA once per d	1 d	↑ MPS rates
Verhoeven *et al.* (2009)[42]	*n* 30, age > 71 years	Test: 3 × 2·5 g Leu three times per d with standardised meal Placebo: standardised meal with wheat flour	3 months	No change in SMM or strength
Wall *et al.* (2013)[35]	*n* 24, age > 74 years	Test: 20·0 g casein with 2·5 g Leu once per d Control: 20·0 g casein without 2·5 g Leu once per d	1 d	Leu co-ingestion = ↑ MPS rates

n, Sample size; EAA, essential amino acids; Leu, leucine; Arg, arginine; ↑, increase; LBM, lean body mass; SMM, skeletal muscle mass.

therefore a significant increase in muscle mass was not observed[44]. Overall, the evidence implies that anabolic resistance may be overcome by increasing the leucine content of a meal. Further studies are warranted and, in particular, should focus on whether long-term leucine supplementation helps reduce loss of muscle mass during ageing.

Table 1 shows studies investigating the impact of various EAA on MPS and muscle protein breakdown in the elderly[34,35,37–39,41–43,45–47].

The role of other amino acids

Research on the role of other AA in MPS during ageing is limited, with the majority of data reported being extrapolated from *in vitro* cell culture models and studies in young pigs. Studies on isoleucine show that it can enhance the fractional synthetic rate of protein synthesis in bovine mammary cells[48], which has important consequences for muscle contraction[49]. Data from a study in pigs, whose diet was supplemented with 7·8 g/kg crystalline lysine, suggest that lysine may potentially function as a regulator of the protein synthesis process, as an increase in total muscle weight and muscle protein accumulation was observed[50]. Lysine was shown to promote protein synthesis at a translational level by helping to construct proteins, and also at a transcriptional level by affecting the expression of myosin in muscle[50]. Tryptophan has been shown to attenuate muscle loss, by directly impacting molecular signalling in skeletal muscle[51] and studies by Wang *et al.*[52] demonstrated that threonine was associated with decreased MPS in young pigs. However, the authors highlighted that the young age of the pigs (25 d) may have contributed to this finding, because the small intestine of young pigs is more sensitive (regarding protein synthesis rates) to dietary threonine levels, and so there may have been a reduced uptake of AA by the

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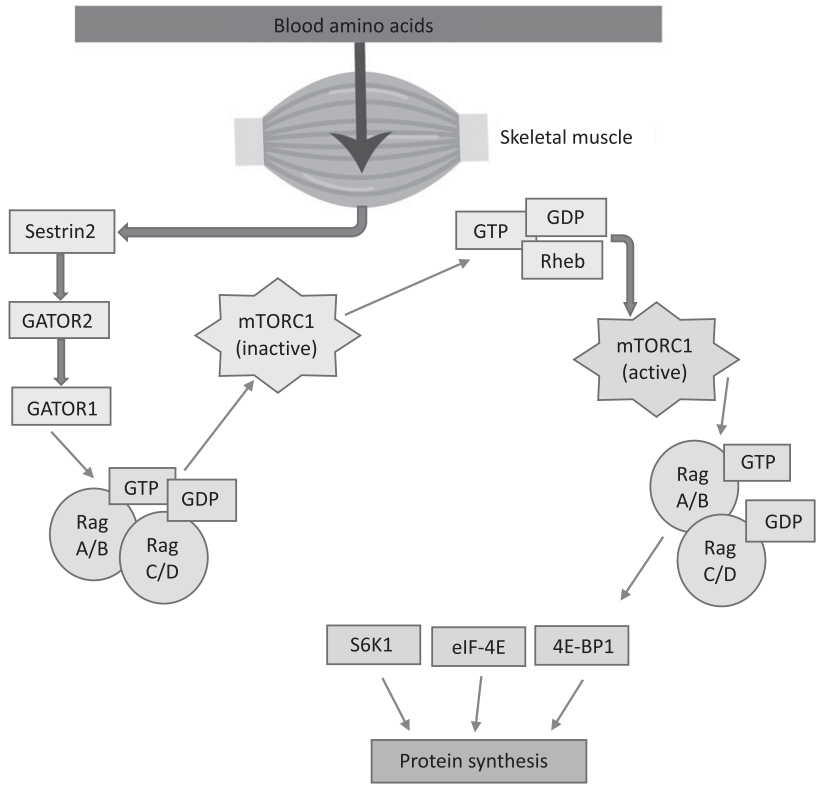

Fig. 1. Overview of mammalian target of rapamycin (mTOR) signalling pathway showing the basic features of mTOR involved in regulating muscle protein synthesis. Amino acids enter the muscle from the bloodstream. Sestrin2 is involved in sensing and signalling the amino acids to the Rag GTPases[31]. The interaction of Sestrin2 with Gap Activity TOward Rags 2 (GATOR2) inhibits mTOR complex 1 (mTORC1) signalling in the absence of leucine[31]. GATOR1 is a Rag GTPase-activating protein which causes RagA/B to switch to an inactive form containing GDP, which inactivates mTORC1 in the absence of amino acids. GATOR1 inhibits mTORC1[29]. In the presence of amino acids, GATOR2 activates RagA/B and inhibits GATOR1, which promotes activation of mTORC1. Rheb is an essential and potent kinase activator of mTORC1[29]. Activation of mTORC1 brings about phosphorylation of 70 kDa ribosomal protein S6 kinase 1 (S6K1) and eukaryotic translation initiation factor 4E-binding protein 1 (4E-BP1), thereby promoting protein synthesis by activating ribosomal protein S6 and by causing the release of eIF-4E, the translation initiation factor[32].

intestinal mucosa, even in the case of excess threonine. While these studies suggest a role for other AA in MPS and preserving muscle, it is important to acknowledge that none of these studies involved aged animals, highlighting the need for further studies in older models to confirm if the effects are similar.

Other factors that influence muscle protein synthesis

Protein intake pattern

While there is no working definition for protein intake pattern, it has been described as the spread of protein over a number of meals (protein spread-feeding), or the consumption of a large amount of protein in a single meal (protein pulse-feeding; PPF)[21]. There is conflicting evidence as to whether protein intake pattern is relevant to protein synthesis and SMM preservation. Kim *et al.*[53] investigated the effects of ingesting protein in older adults as mixed meals of two doses of protein (0·8 g/kg per d or 1·5 g/kg per d) and two intake patterns (uneven; 15, 20 and 65 % protein at each meal: *v.* even; 33 % protein distribution at each meal) over 4 d. The results showed that protein intake above the RDA (1·5 g/kg per d) increased whole-body net protein balance through higher rates of protein synthesis but protein intake pattern had no effect on net protein balance. While there were a number of limitations with this study, namely small sample size (four or five subjects per group), short study duration (4 d) and variable sex ratio, a more recent longitudinal study, involving a larger number of participants (seven subjects per group), also reported that protein intake pattern had no effect on lean body mass, muscle strength or functional outcomes in older adults[54]. As the minimal RDA for protein was provided in all of these studies it would be expected that maximal MPS response would be achieved and thus not affected by protein intake pattern. However, earlier research by Arnal *et al.*[18] tested the efficiency of PPF *v.* protein spread-feeding in improving protein anabolism in healthy elderly women. After 14 d, the PPF group had higher N retention compared with the protein spread-feeding group, due to higher whole-body protein synthesis rates. A more recent study by Bouillanne *et al.*[55] supports this finding, demonstrating that PPF improved MPS in hospitalised (malnourished) elderly patients by increasing plasma postprandial EAA. Additionally, the authors reported that postprandial AA bioavailability persisted after 6 weeks of PPF. While results in undernourished patients[55] cannot be directly extrapolated to healthy adults, these studies suggest that a single high-protein meal may be better at stimulating MPS than protein spreading

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throughout the day. It is repeatedly emphasised that the MPS response to food intake lasts for 4–5 h after ingestion in adults[18,56,57]. Norton *et al.*[58] recently reported that a twice-daily supplementation (at breakfast and lunch) of a milk-based protein matrix (containing 106 (SD 20) g protein per d) increased lean tissue mass in older adults. This may be a more favourable approach for older adults who suffer from reduced appetite and gait speed and poor nutritional status. However, as protein synthesis is not the only controlling factor in muscle protein accretion, it is not possible to conclude that protein consumption at regular intervals throughout the day will maximise daily muscle protein accretion. The current evidence available makes it difficult to confirm that PPF is the superior protein intake pattern. Further studies on the impact of protein intake pattern on MPS in older adults are warranted.

Protein quality

Protein quality, defined by its AA composition and digestibility, has a significant impact on protein metabolism[17] and affects the potential of a protein to contribute to increased MPS following consumption[10]. Studies suggest that animal protein sources are superior to plant sources in their ability to stimulate MPS[59,60] and increase SMM. Phillips[59] reported that protein from beef was superior in its ability to stimulate postprandial MPS in middle-aged men (approximately aged 55 years) compared with an isonitrogenous amount of soya-based protein. Another study in younger men compared the habitual consumption of dairy protein with soya protein, in conjunction with similar resistance exercise regimens, for a period of 12 weeks, and reported an increase in lean muscle mass in the dairy product group[60]. Whey protein is distinct from other proteins because it is rapidly digested and contains a high proportion of EAA[61]. Volek *et al.*[61] demonstrated that whey was significantly ($P<0{\cdot}05$) more effective than an isoenergetic amount of soya protein in increasing lean body mass in young men following a 36-week study that included defined exercising conditions. Overall, studies assessing the ability of soya protein to increase MPS have consistently shown that soya protein is incapable of increasing MPS to the same extent as an isonitrogenous amount of whey protein[62], skimmed milk[63] or beef[50].

Studies involving other plant-based proteins indicate that they can stimulate MPS to a comparable level as dairy proteins but only when consumed in amounts that largely exceed the RDA. This is probably due to the fact that most plant-based proteins have a lower leucine content of 6–8 % compared with animal proteins which have 8·5–10 %, and thus when consumed in low doses, do not increase MPS to a comparable extent as animal proteins[10,64]. However, when matched for leucine content or protein quantity, MPS rates in response to plant proteins do not differ significantly from animal proteins. This was demonstrated in a study by Joy *et al.*[64] who observed that 48 g rice protein decreased fat mass, increased lean body mass, skeletal muscle hypertrophy and muscle strength to a comparable degree as an equivalent amount of whey protein during an 8-week intervention study in healthy young men. In agreement, Babault *et al.*[65] reported that 25 g pea protein resulted in a comparable increase in SMM compared with 25 g whey protein, in a 12-week study involving 137 young males. Data from these studies make it reasonable to suggest that plant proteins can be used as an alternative to animal proteins, but only when the amount consumed is greater than the RDA for protein. To date there have been limited studies performed to extrapolate these findings to older adults. Gorissen *et al.*[66] compared the effects of wheat protein to whey and casein proteins on MPS rates in older adults. Findings revealed that ingestion of 35 g wheat protein hydrolysate, providing 2·5 g leucine, increased MPS rates to a comparable level as intact casein, despite casein resulting in a more prolonged aminoacidaemia. The same study found that although intact whey protein caused a notable increase in plasma EAA concentrations, ingestion of wheat protein hydrolysate caused more sustained levels of AA in the circulation, which resulted in greater stimulation of postprandial MPS rates[66]. The authors suggested that the sustained levels of AA could improve anabolic resistance in an older population, leading to greater MPS response[66]. Interestingly, a recent study in aged rats fed a meal mixed of soya and whey proteins (70 % soya, 30 % whey) reported that plant proteins can counteract anabolic resistance as efficiently as animal proteins, but only if the protein and leucine content are increased[67]. In general, these studies indicate that to achieve comparable effects to animal proteins, plant-based proteins need to be incorporated in the diet at high daily doses. However, further studies in older adults are warranted to confirm the effects in this cohort.

Protein digestibility, absorption kinetics and older adult physiology

Protein digestibility and absorption kinetics are independent predictors of postprandial MPS[68,69]. Protein digestibility relates to the amount of dietary protein that is effectively digested and absorbed in a form acceptable for body protein synthesis[70]. It is influenced by protein structure, the food matrix in which a protein is contained, the presence of anti-nutritional factors and the processing method applied to a food[71]. Digestion speed of a protein can also influence postprandial protein retention and accretion[69,70]. Slowly digested proteins, such as casein, are associated with slower AA absorption kinetics and this affects whole-body protein anabolism by promoting protein deposition[72]. Casein promotes postprandial protein deposition by inhibiting proteolysis without excessive increase in AA concentration in the plasma[72]. Further, the importance of digestion rate on protein deposition in young men has been investigated[69]. It was demonstrated that in this cohort, slow-digesting proteins are better utilised postprandially than fast-digesting proteins, as slow-digesting proteins cause higher postprandial protein gain than fast-digesting proteins[69]. Interestingly, later studies by these authors revealed that fast-digesting proteins may be more beneficial for older adults, as it limits the loss of protein during ageing by affecting protein turnover[73] and protein gain[74]. This is because fast-digesting proteins are associated with increased AA absorption and fast protein turnover[73]. The authors reported a more favourable postprandial leucine balance in elderly subjects with fast-digesting proteins[73]. Data from these studies suggest that older adults should aim to consume more fast-digesting proteins than slow-digesting proteins, in order to limit protein loss during

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ageing, which may favourably make an impact on the maintenance of muscle mass and strength. Further studies are warranted to elucidate the role that protein type may play in muscle mass preservation.

It has been reported that protein digestion rates and subsequent MPS in healthy older adults is comparable with younger adults[75]. However, protein digestion efficiency may be a key determinant of AA bioavailability for protein synthesis[76]. A study of older adults, with reduced ability to chew, reported lower whole-body protein synthesis rates compared with dentate subjects following consumption of meat[77]. Impaired chewing efficiency not only delayed absorption of meat proteins, but also lowered the amount of AA entering peripheral blood. While an increase in plasma AA was observed, there were insufficient levels to stimulate whole-body protein synthesis. These findings highlight the importance of considering chewing efficiency when developing protein-rich snacks and meals for older adults. The food form must be adapted for chewing ability to allow for maximum AA absorption and postprandial protein synthesis. Future research should investigate how protein digestion and absorption kinetics of different proteins sources influence aminoacidaemia and MPS rates while taking into account the chewing efficiency of its subjects.

Protein source. Animal proteins, such as those sourced from eggs, dairy products and meat, are highly digestible (>90 %)[78]. Studies have reported a fast and transient increase in plasma AA levels following consumption of whey protein by healthy adults[69,72] and additional studies revealed that 50–70 % of protein-derived AA from milk and beef become bioavailable 5–6 h after consumption[73,76]. Once bioavailable, these AA can help increase postprandial MPS. Plant protein sources (such as oat, pea, potato) exhibit digestibility values ranging from 45 to 80 %[78]. The lower digestibility rates associated with plant proteins may be due to the presence of anti-nutrients, such as phytates, tannins and saponins[78]. *In vivo* studies with pigs demonstrated that polyphenols (tannins in particular) decreased ileal protein digestibility by 5 %[79]. More recently Dufour *et al.*[80] reported that the release of polyphenols from a food matrix had a negative impact on protein digestibility. In the study, when proteins were consumed with phenolic extracts protein digestibility was decreased by 15 % compared with when the proteins were consumed with whole fruit and vegetables that contained polyphenols[80]. These studies indicate that the composition of a food matrix, in particular the polyphenol content, has an impact on plant protein digestibility. Future strategies to improve the digestibility and absorption kinetics of plant proteins, and subsequently MPS, could be selective plant breeding[10] that considers polyphenol composition or foods designed based on specific protein:polyphenol content. Further studies, however, are needed to identify optimal protein:phenol ratios.

It has been reported that plant proteins, particularly soya and wheat, have a lower potential to stimulate MPS because their AA are readily converted to urea[64,81–83]. It is thought that the rapid conversion of plant proteins to urea is due to a lack of one or more EAA[10]. This unbalanced AA profile may lead to a lower retention of AA by the digestive tract and thus cause more AA to enter the portal vein to hepatic tissue, which signals the liver to increase ureagenesis[10]. The increased conversion of AA to urea means that less plant-derived AA become available in the systemic circulation, resulting in lower postprandial availability of AA to stimulate MPS[10]. Currently there is a lack of research on the digestion kinetics of plants other than soya and wheat, and it is not fully understood why consuming excessive amounts of plant proteins, which do have a complete AA profile, increases urea production; therefore future research in this area would be of interest.

Food processing and protein digestibility. Food processing also makes an impact on the digestibility and absorption kinetics of proteins. Glycation, also known as the Maillard reaction, is the chemical reaction that occurs between AA and reducing sugars, usually in the presence of heat. A recent systematic review on the effect of processing on milk protein digestibility concluded that glycation decreases digestibility[84]. Both *in vitro* and *in vivo* studies have reported that AA glycation can have an impact on protein digestibility and the bioavailability and functionality of AA[85–88]. It is hypothesised that glycation decreases protein digestibility by: (1) directly blocking lysine and preventing it from cleavage by digestive proteases; (2) indirectly blocking lysine residues which are near the enzyme cleaving sites; and (3) causing cross-linking which hinders the accessibility of the cleavage sites by proteases[84]. The high temperature used in processing can cause protein denaturation. However, this does not negatively make an impact on digestibility, but rather alters the gastric emptying of proteins, affecting digestion kinetics[84]. Other processing reactions can also affect digestibility; for example, oxidation has been shown to modify EAA such as methionine, tyrosine and tryptophan[89]. Furthermore, racemisation, which transform L-amino acids into their D-form, is thought to affect ileal protein digestibility by reducing recognition of D-amino acids and free D-amino acids by peptidases[90]. Cooking significantly increases the protein digestibility of chickpeas, beans, lentils, peas and kidney beans[91]. Microwave cooking, using a very low amount of energy (500 J), significantly increased the protein digestibility of faba bean varieties from 46·0, 52·2 and 51·5 %, to 57·1, 68·0 and 53·2 %[92]. Pressure cooking and autoclaving caused significant improvement in protein digestibility in moth beans[93], soyabeans[94] and peas[95]. In light of all this evidence, the metabolic impact of food processing methods on SMM remains to be elucidated. It is clear that processing affects protein digestibility, but most studies do not report the impact of food processing on MPS or SMM; thus further research is warranted in this area.

Food strategies to improve muscle protein synthesis in older adults

Protein blends

A protein blend is the combination of two or more protein sources and the potential of protein blends to increase MPS has been the focus of recent research[96–98]. Witard *et al.*[16] suggested that protein blending has the potential to allow for optimal plasma aminoacidaemia to increase and lengthen the MPS response[16]. AA in a protein blend can potentially act synergistically to bring about increased MPS response and biological activity. Blending different protein sources could also potentially

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improve protein digestion rates to a comparable level to that of fast-digesting proteins, which is beneficial for maximum MPS.

The efficacy of a protein blend for MPS stimulation in young adults has been evaluated, and it was found that 19·3 g of a protein blend that consisted of 25 % soya, 25 % whey and 50 % casein prolonged postprandial plasma aminoacidaemia to a level comparable with 17·7 g whey protein[96]. Likewise, Borack *et al.*[97] used the same protein blend in a study with elderly male subjects and reported similar responses in hyperaminoacidaemia, mTORC1 signalling and MPS stimulation. In both studies the protein diets (protein blend *v.* whey) were matched for total EAA but not leucine content, although both diets still contained enough leucine to stimulate MPS[99]. While both protein diets elevated hyperaminoacidaemia and mTORC1 signalling, fractional synthetic rate elevation was sustained to a significantly ($P<0·05$) greater extent 2–4 h postprandially in the whey protein group[97]. In addition, the study reported that the protein blend did not extend the duration of MPS stimulation more than one individual protein source (whey)[97]. This re-emphasises the idea that whey protein is more efficient at promoting positive N balance in skeletal muscle after exercise than soya–dairy blends given at the same dose. An *in vivo* study by Butteiger *et al.*[98] reported that a soya–dairy blend meal with different ratios of dairy:soya proteins (25 % whey, 50 % casein, 25 % soya) significantly ($P<0·05$) increased MPS rates to a greater extent compared with whey or soya protein alone. The results were attributed to the increased casein level used in the blend, and the slow-digesting nature of this protein which delays the appearance of plasma AA. It is important to acknowledge that the study was performed in young rats; thus trials in older models should be conducted to see if a similar response would be observed. Also, it remains to be explored if these findings would influence muscle wasting conditions.

Collectively, the data from these studies demonstrate that protein blends can be designed to increase MPS and potentially attenuate muscle wasting conditions. However, all of these studies utilised a mixture of plant and animal proteins, and all contained dairy proteins. There is limited research on the biological impact of plant-only protein blends. Van Vliet *et al.*[10] suggested that the consumption of plant-based protein blends (regardless of leucine content), with no limiting AA, may have a more positive impact on postprandial MPS response than ingestion of an individual plant protein source in older adults and compromised populations, but additional studies are warranted. For example, *in vitro* synergy tests to compare the effects of plant protein blends to individual plant or animal protein sources are required, and additional *in vivo* studies investigating the impact of plant-based protein blends on muscle mass gain and muscle loss are also lacking. It is also necessary to explore what benefits could be achieved from using protein blends with high leucine content[67] and what quantities of plant-based protein blends should be consumed by older adults with anabolic resistance.

Food form

Liquid- and solid-food matrices can elicit different postprandial aminoacidaemia responses. The impact of food form on protein delivery to the muscles to enhance MPS rates has been the focus of recent research. Conley *et al.*[100] provided beverage and solid-form (macronutrient-matched) meal replacements to older adults and reported that the liquid form resulted in higher initial (30 min) and sustained (4 h) plasma AA concentration compared with the solid-form supplement. Burke *et al.*[101] reported that liquid forms of protein (soya milk, skimmed milk) achieved higher peak concentrations of plasma AA compared with ingestion of solid protein-rich foods (protein bar, beefsteak, eggs); however, this study was carried out in young adults. This limited evidence suggests that liquid-form protein-rich meals elicit more rapid and higher plasma AA concentration, provided they contain sufficient amounts of EAA, but further studies in older adults are needed. Furthermore, Stull *et al.*[102] found that liquid meal replacement products were less satiating than solid meal replacements in older adults, which implies that liquids may be better to increase protein intake and increase MPS rates in undernourished individuals, where an increase in overall food intake is required[23], but solid meals with more satiating properties may be preferable when food intake is more restricted.

Snack foods

The term 'snack' lacks a consistent definition. 'Snacking' has been described as an eating occasion involving the consumption of energy and nutrients, regardless of whether healthy options are chosen[103]. Other definitions refer to 'snacks' as typically high-energy, sugary, salty or fatty food or beverage items[103] and define a 'snack' based on the time it is consumed during the day[104–107] or based on the amount consumed[108]. The lack of a concise definition for snack foods makes it difficult to classify 'snacking' as beneficial or harmful to health. Indeed, many studies tend to focus on the detrimental effects of snack foods and fewer studies focus on the potential positive impact. For older adults the use of 'snack foods' could play a role in promoting healthy ageing, especially for undernourished subjects. Inadequate protein and energy consumption in older adults can contribute to decreased functional ability, resulting from increased loss of muscle mass, insufficient energy stores, and decreased immune function[109]. Snacking can increase daily protein intake in older adults[110], and subsequently potentially improve muscle mass preservation and/or muscle mass gain. Zizza *et al.*[25] reported a significant increase in energy, protein, carbohydrates and total fat in 'snacking' older adults (≥ 65 years) compared with non-snacking older adults[25]. The authors reported that 'snacking' older adults consumed 6 g more protein than 'non-snacking' older adults[25]. While the study did not assess the relationship between increased protein intake and MPS or muscle mass preservation in the test subjects, it is reasonable to extrapolate that the additional dietary protein positively influenced MPS and muscle mass gain. Snacking is also positively associated with gait speed[111] and recent research has shown that an even distribution of protein intake throughout the day can positively influence frailty in older adults and quality of life[22]. Physical function is important in this age group, as increased frailty increases the risk of falls, which in turn increases the risk of mortality and morbidity[19].

Other benefits associated with snacking include the fact that increased meal frequency has the potential to improve lipid

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profiles and subsequently decrease the risk of CVD, by decreasing total and LDL-cholesterol[103]. Furthermore, an inverse relationship between snacking and body weight has been reported[103], suggesting a potential role for snacking in maintaining a healthy body weight in older adults. These studies suggest that snacking has the potential to improve nutritional status and physical function; however, knowledge on the role of snacks in preserving muscle mass is limited. Further research is warranted to evaluate the role of snacking in overcoming anabolic resistance in the elderly and in muscle mass preservation.

Conclusion

To date, leucine remains the only EAA that has been extensively studied for its role in MPS in older adults, with the majority of studies indicating that incorporating leucine in sufficient doses in the diet increases MPS and improves SMM in this cohort. Additional studies are needed to investigate the role of other EAA, in particular isoleucine, lysine, threonine and tryptophan. There is still no consensus as to whether protein intake pattern can significantly affect MPS, but studies relating to older adults suggest that health and nutritional status are important considerations when deciding which intake pattern is most appropriate. The digestibility and absorption kinetics reported for plant proteins suggests that they have inferior anabolic properties for stimulating MPS, compared with animal protein sources. However, blending different protein sources has had favourable effects on MPS; thus, this strategy has potential. Studies are needed to identify optimal protein blends, including plant protein blends that stimulate MPS and investigate their potential to preserve SMM in older adults. Liquid-form foods have a more favourable effect on postprandial aminoacidaemia than solid-form foods; however, the effects of food form on MPS and SMM preservation need to be further elucidated. The evidence supporting snack foods as a strategy to improve MPS and SMM is currently limited; however, snacking may be beneficial as a strategy to increase protein intake and maintain a healthy body weight for undernourished older adults, and thus would help to promote healthy ageing.

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