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Nutrient-dense protein as a primary dietary strategy in healthy ageing: please sir, may we have more?

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A progressive decrement in muscle mass and muscle function, sarcopoenia, accompanies ageing. The loss of skeletal muscle mass and function is the main feature of sarcopoenia. Preventing the loss of muscle mass is relevant since sarcopoenia can have a significant impact on mobility and the quality of life of older people. Dietary protein and physical activity have an essential role in slowing muscle mass loss and helping to maintain muscle function. However, the current recommendations for daily protein ingestion for older persons appear to be too low and are in need of adjustment. In this review, we discuss the skeletal muscle response to protein ingestion, and review the data examining current dietary protein recommendations in the older subjects. Furthermore, we review the concept of protein quality and the important role that nutrient-dense protein (NDP) sources play in meeting overall nutrient requirements and improving dietary quality. Overall, the current evidence endorses an increase in the daily ingestion of protein with emphasis on the ingestion of NDP choices by older adults.

Muscle mass: Sarcopoenia: Protein quality: Diet quality

Sarcopoenia impacts a substantial portion of older adults and is characterised by a progressive loss of skeletal muscle mass and function at rates of 1–2 and 1·5–5 % per year, respectively[1,2]. A plethora of adverse health outcomes accompany sarcopoenia, including an increased risk of mobility impairments[3], falls[4], dependent living[5], metabolic disease[6], hospitalisation[7] and premature mortality[8,9]. Annually, the cost of hospitalisation for sarcopoenic individuals is about $2300 higher than non-sarcopoenic individuals and totals about $40 billion in the United States alone[7]. Moreover, the impacts of sarcopoenia will continue to escalate as the proportion of the population that is older increases. Feasible, low-cost and effective strategies are clearly needed to mitigate the effects of sarcopoenia.

One component of sarcopoenia is reduced skeletal muscle mass[1,2]. Skeletal muscle mass is regulated in part by fluctuations in muscle protein balance[10]. Muscle protein balance is the difference between muscle protein synthesis (MPS) and muscle protein breakdown, such that skeletal muscle can be in a net anabolic (MPS > muscle protein breakdown) or net catabolic (MPS < muscle protein breakdown) state where proteins would be accrued or lost, respectively[10,11]. Resistance exercise (RE) and dietary protein stimulate MPS independently and synergistically promote temporary increases in

Abbreviations: DIAAS, digestible indispensable amino acid score; EAR, estimated average requirement; GFR, glomerular filtration rate; IAAO, indicator amino acid oxidation; IAA, indispensable amino acid; MIP, multi-ingredient protein; MPS, muscle protein synthesis; MyoPS, myofibrillar protein synthesis; NDP, nutrient-dense protein; PDCAAS, protein digestibility-corrected amino acid score; RE, resistance exercise.

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skeletal muscle anabolism[6,12–15]. In younger adults, muscle mass typically remains relatively stable because the meal- and loading-induced fluctuations between muscle anabolism and catabolism are in equilibrium[13]; however, in older adults, the chronic persistence of net muscle catabolism contributes to the progression of sarcopoenia[11,16]. One of the main sources of this persistent age-related muscle catabolism has been referred to as anabolic resistance[17,18], which is a term coined to describe a blunted stimulation of MPS seen in older persons *v.* younger persons at the same ingested protein dose. Importantly, older adults do mount an MPS response comparable in magnitude to that of younger adults with larger doses of protein[18]. Thus, regular practice of RE and higher than recommended dietary protein intakes likely represent the most effective countermeasures to sarcopoenia[12,19].

The quality and amount of dietary protein are key factors when optimising protein nutrition, and research continues to refine protein recommendations for older adults[20,21]. In addition to calls for increasing protein intake, researchers have started exploring multi-ingredient and whole-food protein sources[16,22]. Whole-food sources can provide high-quality protein and several additional nutrients necessary for healthy ageing[16]. We discuss the quality and dose of dietary protein as it pertains to healthy ageing. Moreover, we examine the thesis that high-quality, nutrient-dense proteins (NDP) are a cornerstone of high-quality diet and may aid countering sarcopoenia. Lastly, we provide recommendations for older adults to improve their protein nutrition and promote retention of skeletal muscle and function with ageing.

Protein quality

Dietary proteins provide amino acids for several metabolic functions in the human body, and the synthesis of new skeletal muscle proteins is an important metabolic fate of amino acids from proteins ingested in each meal[23]. Different dietary proteins have been studied, and several ways have been established to differentiate and classify dietary proteins' capacity to provide amino acids for tissue growth and remodelling[24]. Recommendations for dietary protein ingestion have focused on protein quantity and quality. Specifically, protein quality represents the property of a given protein to provide the amino acids needed to fulfil human physiological requirements based on its digestibility and amino acid profile[25–27]. Therefore, proteins are scored differently for quality according to the abundance of indispensable amino acids (IAA), and the digestibility of the protein following its ingestion[20,25,26].

The protein digestibility-corrected amino acid score (PDCAAS) was traditionally utilised to assess protein quality. PDCAAS has been used since 1991 when the FAO of the UN recommended the use for the evaluation of protein quality in food products: Protein Quality Evaluation: Report of Joint FAO/WHO Expert Consultation[28]. PDCAAS is calculated by multiplying the amino acid content score of a crude protein by its digestibility defined by faecal losses. Nevertheless, PDCAAS has been noted to have several limitations[26,28,29]. For instance, PDCAAS is truncated at a score of 1 with the rationale that protein needs to optimise growth cannot be greater than that provided by the gold standard protein. Therefore, different high-quality proteins might receive the same score. For example, whey protein and soya protein isolate both have a PDCAAS score of 1·0, despite significant differences in their amino acids composition and availability[26,29] that would have them with untruncated PDCAAS scores of 0·97 and 1·12, respectively. Furthermore, the use of faecal-derived protein digestibility values is now known to be problematic because it does not account for amino acid metabolised by the gut microbiota. These factors are frequently a source of overestimation for the quality of some proteins[26,28,29].

Because of the limitations inherent to the PDCAAS system, the digestible indispensable amino acid score (DIAAS) was recommended in a 2013 report published by an FAO Expert Consultation on Protein Evaluation in Human Nutrition. DIAAS addresses several limitations of the PDCAAS, and it is calculated considering the most limiting IAA in a dietary protein when expressed relative to a protein containing amino acids that are in-line with the age appropriate amino acid scoring pattern. When acquiring data for DIAAS percentage calculation, true ileal digestibility data from human subjects would be preferable; however, because collecting ileal samples is difficult in human subjects, pigs have been the primary model to generate the ileal digestibility data, although rats are also an acceptable model[28,29].

The requirements for each particular IAA vary during human lifespan. DIAAS values are calculated based on the estimated average requirement (EAR) for different amino acids for three age ranges: birth to 6 months, 6 months to 3 years and >3 years[28]. Cut-off values for DIAAS are commonly used to classify proteins. A DIAAS score <75 are considered to be low-quality proteins, 75–99 are considered good quality and ≥100 are high/excellent quality[28]. Further information on the use of DIAAS and limitations are covered elsewhere[20,25,27]. Table 1 compares DIAAS and PDCAAS values for different proteins and food sources.

In addition to total IAA content, leucine content is a particularly important determinant of protein quality for maintaining skeletal muscle, especially for older adults[26]. Briefly, leucine is the key amino acid that stimulates MPS[30]. Although, all IAA are needed to sustain the MPS response[31], leucine is the main activator of MPS[30]. Corroborating such assumptions, studies from our group have shown that leucine content per meal is the primary variable leading to MPS activation[32–34]. However, ageing seems to be linked to a decreased sensitivity to the stimulatory effect of leucine on MPS[35]. Consequently, older subjects might need to ingest more leucine to activate MPS[34,36–39]. Recent advances in protein quality scoring and amino acid metabolism provide support for increasing protein requirements in older populations and focusing on higher quality proteins[33,34,39,40].

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Table 1. Quality scores (DIAAS and PDCAAS) for different proteins and the respective limiting amino acids

Protein or food source	DIAAS	PDCAAS	Limiting AA
Animal-derived protein			
Whole milk powder*[a]	1·43	1	Met + Cys
Skimmed milk powder*[b]	1·23	1	Met + Cys
Milk protein concentrate*[b]	1·41	1 (1·25)	Met + Cys
Whey protein isolate*[b]	1·25	1 (1·12)	His
Whey protein concentrate*[b]	1·33	1	His
Bovine muscle hydrolysate*[c]	0·81	–	Trp
Bovine collagen hydrolysate*[c]	0	–	Trp
Chicken breast*[a]	1·08	1	Trp
Egg (hard-boiled)*[a]	1·13	1	His
Bacon (cooked)*[d]	1·42	–	Val
Ham (conventionally cured)*[d]	1·26	–	Val
Pork loin (cooked 72°C – well done)*[d]	1·17	–	Val
Vegetal-derived protein			
Soya protein isolate B#[e]	0·90	1 (0·97)	Met + Cys
Soya protein isolate A#[e]	0·90	0·95	Met + Cys
Pea protein concentrate#[e]	0·82	0·89	Met + Cys
Rice protein concentrate#[e]	0·37	0·42	Lys
Cooked peas#[e]	0·58	0·60	Met + Cys
Cooked kidney beans#[e]	0·59	0·65	Met + Cys
Cooked rice#[e]	0·60	0·62	Lys
Cooked rolled oats#[e]	0·54	0·67	Lys
Wheat bran#[e]	0·41	0·53	Lys
Roasted peanuts#[e]	0·43	0·51	Lys
Maize-based breakfast cereal#[e]	0·01	0·08	Lys
Brown rice#[f]	0·42	–	Lys
Polish rice#[f]	0·37	–	Lys
Buckwheat#[f]	0·68	–	Met + Cys
Oats#[f]	0·43	–	Lys
Dehulled oats*[g]	0·51	–	Lys
Whole wheat[f]	0·20	–	Lys
Wheat*[g]	0·43	–	Lys
Yellow dent maize*[g]	0·48	–	Lys
Nutridense maize*[g]	0·54	–	Lys
Rye*[g]	0·47	–	Lys

AA, amino acids; Cys: cysteine; DIAAS, digestible indispensable amino acid score; His, histidine; Lys: lysine; Met: Methionine; Trp: Tryptophan; Val, valine. PDCAAS, protein digestibility-corrected amino acid score.
*DIAAS percentage >3 years Older children, adolescents, and adults. Values in brackets are examples of actual untruncated PDCAAS scores.
#DIAAS percentage 6 months to 3 years old child.
a(28), b(152), c(148), d(151), e(29), f(150), g(149).

Plant-based and animal-based proteins

Animal-based proteins (i.e. primarily milk-derived) have typically been studied as a primary protein source to stimulate MPS in older adults[16,41]. Most animal-source proteins, and milk proteins in particular, generally have higher digestibility and no limiting amino acids lower than the age-specific scoring pattern[29]. Despite the strong evidence that the ingestion of animal-based proteins effectively promotes muscle anabolism, there have been shifting attitudes in consumers seeking plant-based proteins on the basis of environmental sustainability and population health benefits[42].

Compared to animal protein, most plant-based proteins, when ingested as foods, have a lower digestibility due to the presence of dietary fibre and anti-nutritional compounds that inhibit enzymatic protein digestion[43]. Furthermore, most plant-based proteins typically have a lower leucine content or are limiting in at least one IAA (often lysine, methionine or cysteine)[44]. In line with these characteristics, the ingestion of isolated soya protein, which is considered a high-quality plant-based protein[44], stimulated MPS to a lower degree when compared with an isonitrogenous dose of beef[21] and whey[45] protein in older adults. In addition, 40 g soya protein only mildly stimulated MPS in older adults[45]. When comparing wheat protein with casein, which is a slowly digested protein containing lower leucine than whey, only casein promoted an increase in MPS in older adults[46]. Nonetheless, the consumption of 35 g wheat protein and 35 g casein resulted in a similar plasma leucine concentration during the postprandial period[46]. Furthermore, to promote MPS with the consumption of wheat protein in these elderly subjects, a considerable dose of wheat protein (60 g), which is leucine-matched amount with 35 g whey, had to be consumed[46]. This is relevant, since consuming larger doses of dietary protein than usual may not be feasible for older adults who may have a lack of appetite or have an impaired digestive capacity.

The use of protein blends (animal + plant-based protein or different plant-based proteins) has been considered as an approach to compensate for the lower anabolic potential of plant-based protein[47]. For instance, consuming 19 g of a mixture containing 50 % sodium caseinate, 25 % whey and 25 % soya protein isolate, 1 h after high-intensity exercise (one bout) and immediately after RE for 12 weeks (long term), promoted similar muscle anabolism in young men compared to the ingestion of 18 g whey protein[48,49]. However, the same research group reported that there was no increase in MPS following the ingestion of 30 g of the mixture after RE in older adults, although phosphorylation of proteins critical in stimulating the MPS pathway was increased[50]. Theoretically, fortifying plant-based protein with leucine is another strategy to correct the low content of leucine and potentiate MPS stimulation[34]. To our knowledge, only one study has determined the MPS response to the ingestion of leucine-enriched plant-based protein[51]. Churchward-Venne *et al.*[51] showed no additional muscle anabolic response to the ingestion of 20 g leucine-enriched soya protein after RE compared to the same dose of whey and soya protein in young men. More studies are needed to determine the effect of ingesting leucine-enriched plant-based protein on muscle anabolism in older adults.

The consumption of plant-based protein sources such as blends of different proteins[50], or plant-based protein isolates (i.e. wheat, potato or pea protein)[46,52] have proven to be potential alternatives to animal-based protein in young adults. To date, however, the consumption of such protein blends and their effects on muscle anabolism in older adults are unclear. Furthermore, to our knowledge, no study has determined the MPS response to whole meals containing vegetarian *v.* non-vegetarian food as opposed to isolated proteins. Therefore, further research is needed to find a practical dietary strategy

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balanced for environmental sustainability and healthy ageing in older adults.

High-quality, nutrient-dense protein

Most of the knowledge regarding protein ingestion and MPS comes from studies testing isolated proteins[12,22]. These findings have contributed substantially to our understanding of protein metabolism, however, they lack some ecological validity, since human subjects do not often consume isolated protein sources[22]. Therefore, investigating MPS following the ingestion of protein and additional nutrients, such as whole-foods or multi-ingredient supplements, represents a blossoming area of research. Different protein sources and food matrices might present distinct dynamics when considering the speed of digestion, bioavailability and the consequent increment in blood amino acid levels[53,54]. Co-ingestion of carbohydrates with protein can modulate protein digestion and absorption kinetics[55], and the co-ingestion of lipids with protein may slow gastric emptying rates affecting amino acid absorption, thereby changing the MPS response[56]. Such studies are relevant because various characteristics and dietary components of the protein source may contribute to the regulation of MPS[22] and result in proteins surpassing the expected MPS response when considering only protein quantity[57]. Especially for the elderly population, the MPS response to ingesting protein with several additional nutrients or whole-food is highly relevant[54].

The term NDP refers to the package of nutrients, for example calcium, iron and vitamin D, that are often co-consumed with sources of protein[16,22]. These additional nutrients may positively influence MPS, muscle mass or contribute to improving dietary quality and thus have effects on other health-related outcomes[16,54,58–61]. Research advancing knowledge regarding nutrient interactions, bioavailability and modulation of the sensitivity to the effect of IAA in the muscle are essential to optimise dietary protein recommendations and nutritional supplements[22].

One strategy that has been tested as a means to increase the anabolic capacity and nutrient density of meals or nutritional supplements is by changing the leucine content of the meal[33,34,62]. We tested if adding 5 g crystalline leucine to each main meal would have any impact on rates of MPS over 3 d in older subjects[62]. We found that the additional leucine amplified MPS, and this effect was independent of whether older subjects consumed 0·8 or 1·2 g protein/kg/d[62]. Also, we have shown that the ingestion of 10 g milk protein enriched with 3 g leucine produced a similar myofibrillar protein synthesis (MyoPS) response in comparison with 25 g whey protein isolate containing 3 g leucine[34]. These results show that leucine content is one valuable strategy that can be used to improve nutrient density and optimise the MPS response to regular meals or smaller doses of protein in older adults. Although the exact amount of leucine necessary to maximise MPS in older subjects is unknown[63], we propose that evidence supports the recommendation for consuming at least 2·5–3·0 g per meal[33,34,62].

Another strategy to provide protein and other nutrients relevant to the elderly population is the use of multi-ingredient protein (MIP) supplements[58,64]. MIP often contains vitamins (e.g. vitamin D), minerals (e.g. calcium), PUFA (e.g. *n*-3 fatty acids), creatine and high-quality proteins (i.e. whey and caseinate)[64,65]. Our group has shown that older men ingesting an MIP containing whey protein, creatine, vitamin D and *n*-3 PUFA experienced a 30 % higher MyoPS in the first 24 h after RE when compared to the control group[64]. Moreover, the ingestion of a similar MIP twice daily for 20 weeks has been proven to be an efficient strategy to increase muscle mass and strength in the absence or presence of a RE programme[65]. A recent systematic review and meta-analysis of eight trials concluded that the ingestion of MIP enhances fat-free-mass and possibly strength gains in older individuals involved in RE training[58]. Therefore, MIP, although not food *per se*, could contribute as an additional source of NDP in place to be prescribed to elder populations aiming to prevent muscle mass loss or improve muscle mass and strength gains when performing RE.

Nutritional supplements are a valuable strategy to provide under-consumed nutrients or supplemental compounds such as creatine. Nonetheless, the use of whole-foods is recognised as a preferred approach when considering nutritional recommendations at a population level[16,22]. Milk, dairy, meat and eggs are common whole-food but nutrient-dense sources of protein[16,22,66]. A list of studies testing the direct effect of whole foods on MPS is presented in Table 2. Elliot *et al.*[57] tested the effects of milk on leg protein balance after RE in young subjects. In this study, the authors found that whole milk (8·0 g protein) produced a higher leg phenylalanine and threonine uptake (a proxy for MPS) when compared to fat-free milk (8·8 g protein) and even isoenergetic fat-free milk ingestion (14·5 g protein)[57]. This finding is interesting since the presence of fat in the whole milk might have been expected to negatively impact digestion time, amino acid absorption and, consequently, the anabolic response. Besides milk, beef has also been tested for its effects on MPS. Symons *et al.*[67] showed that young and old subjects respond equally to ingestion of 113 g lean beef when analysing mixed MPS. Symons *et al.*[68] also showed that this mixed MPS does not seem to be further increased when ingesting a 3-fold higher portion of lean beef (340 g *v.* 113 g) independent of age[68]. In addition, Burd *et al.*[69] showed that the overall MyoPS (2–5 h after ingestion) was the same when comparing the ingestion of 30 g protein as fat-free milk or 158 g minced beef in young subjects. It is notable that the MyoPS response to milk was higher during the first 2 h after ingestion[69]. Furthermore, age seems not to diminish the MPS response to an RE bout followed by a protein-rich lean beef meal (340 g lean beef; 90 g protein)[70]. Also, Pennings *et al.*[71] reported that ingestion of minced beef or a beef steak (135 g meat) produced the equivalent MPS responses in older adults. Therefore, the form of

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Table 2. Studies investigating muscle protein synthesis (MPS) response following nutrient dense protein (NDP) ingestion

Study	Subjects (*n*, sex)	RE	Protein source (and quantity)	Measurement of MPS (i.e. infusion, ingestion, intrinsically labelled, etc.)	Time after ingestion (min)	Main results
Elliot *et al.*[57]	6M/2F 3M/5F	Y	237 g whole milk (8g protein) 393 g fat-free milk (14.5 g protein)	2-pool	240	Whole-milk increased utilisation of AA for MPS
Symons *et al.*[67]	Young (5M/5F) Elderly (5M/5F)	N	113 g lean beef (about 30 g protein)	Constant infusion L-[ring-$^{13}C_6$] phenylalanine	300	Ageing does not impair MPS following NDP consumption
Symons *et al.*[68]	Young (8M/9F) Elderly (10M/7F)	N	113 g or 340 g of lean beef (30 g and 90 g protein respectively)	Constant infusion L-[ring-$^{13}C_6$] phenylalanine	300	Protein dose >30 g does not increase MPS
Symons *et al.*[70]	Young (3M/4F) Elderly (3M/4F)	Y	340 g lean beef (90 g protein)	Constant infusion L- [ring-$^{13}C_6$] phenylalanine	300	Ageing does not impair MPS following RE and large dose of NDP
Pennings *et al.*[71]	Elderly (10M)	N	135 g of ground beef or 135 g of beef steak (both 26 g protein)	L-[1-^{13}C] phenylalanine-labelled beef, constant infusion L-[ring-$^{2}H_5$] phenylalanine and L-[ring-$^{2}H_2$] tyrosine	360	Minced beef digested and absorbed quicker; however, no MPS increase
Burd *et al.*[69]	Young (12M)	Y	158 g ground beef patty (30 g) or 350 ml skim milk enriched with whey and casein (30 g protein)	L-[1-^{13}C] phenylalanine-labelled beef/ milk, constant infusion L-[ring-$^{2}H_5$]phenylalanine and L-[ring-3,5-$^{2}H_2$]tyrosine	300	Both beef and milk increased MPS, augmented MyoPS during earlier postprandial stage with milk
Beals *et al.*[72]	Healthy(5M/5F) Overweight (5M/ 5F) Obese adults (5M/5F)	N	170 g ground pork patty (36 g protein)	Constant infusion L-[ring-$^{13}C_6$] phenylalanine	300	Diminished MyoPS following NDP in overweight and obese adults
van Vliet *et al.*[74]	Young (10M)	Y	Whole eggs or egg-whites (18 g protein)	L-[5,5,5-$^{2}H_3$]leucine-labelled whole eggs, constant infusion L-[ring-$^{2}H_5$]phenylalanine and L-[1-13C]leucine	300	Greater MyoPS increase following whole egg ingestion

AA, amino acids; F, female; M, male; MyoPS, myofibrillar protein synthesis; RE, resistance exercise; Y, yes; N, no.

beef, whole or minced, seems to be an irrelevant variable when considering MPS in old subjects. It is noteworthy that factors such as the presence of obesity might produce alternative results since Beals *et al.*[72] showed that young overweight and obese individuals were resistant to the MyoPS response elicited by the ingestion of an NDP meal consisting of 170 g pork when compared to non-obese subjects.

Eggs are another NDP source available to several populations, especially for the elderly[16,73]. The ingestion of whole eggs or egg-whites (both 18 g protein) were compared in a recent study, with a focus on the post-RE MPS response in young subjects[74]. This study showed that MPS was higher when whole eggs were ingested and compared to the ingestion of egg-whites[74]. Such findings add to the previous body of evidence showing that whole foods are efficient, or even more efficient, options to stimulate MPS[57,74]. One exciting aspect of this study[74] is that blood leucine concentrations were the same in both groups during the protein metabolism measurements. Also, whole-body protein metabolism showed no differences between the ingestion of whole-eggs or egg-whites[74]. Therefore, the combination of different nutrients in whole eggs seems to produce a synergetic effect on MPS when compared to egg-whites[74]. The fact that ingesting whole eggs produces a higher MPS response is intriguing, and future research might explore the effects of the several components present in whole eggs on MPS[74,75]. Furthermore, future research could also investigate the effects of eggs specifically engineered with different nutrient compositions, since interventions in hens (i.e. nutritional or intentional strain selection) can profoundly influence the nutritional composition of eggs[76]. Therefore, muscle anabolism induced by ingesting whole-foods, unlike protein isolates, could have some unique characteristics as a result of the whole food matrix or nutrient and non-nutrient components interactions[77,78]. NDP, such as those discussed herein, should be a focus of future research, since few studies investigating these protein sources have evaluated MPS, particularly in older populations.

Ageing and protein requirements

Consuming enough protein is vital for healthy ageing. Since a landmark meta-analysis of nitrogen balance studies[79], the Food and Nutrition Board of the Institute of Medicine has reported the EAR and RDA for protein to be 0·66 g/kg/d and 0·80 g/kg/d, respectively, for

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individuals over 18 years old[80]. The EAR is defined as the lowest intake of good-quality protein necessary for 50 % of individuals to maintain nitrogen balance (i.e. protein mass), and the RDA is two population-derived standard deviations above the EAR, such that most (about 98 %) individuals consuming the RDA for protein will maintain nitrogen balance[80]. The RDA thus represents the minimum protein intake necessary to prevent protein loss and not an optimal intake to support amino acid-requiring processes[22,24,38]. However, it has been questioned if the RDA is indeed sufficient to maintain nitrogen balance in older adults[24,63,81]. Using various measurement techniques, several experts in the field have put forward the thesis that the RDA requires reassessment, since protein intake above the RDA appears necessary to maintain health, particularly in older adults[36–38,63,82–84]. Although some scientific organisations and health ministries have increased their RDA (or respective equivalent) for protein[85,86], protein requirements in most countries remain largely unchanged from values now almost 40–50 years old.

Current measurements: considering whole-body protein balance

The current protein requirements were determined by Rand *et al.*[79] in a meta-analysis of nineteen nitrogen balance studies. Interestingly, the median protein requirement for older adults (based on only a small number of older adults) was 0·17 g/kg/d more than younger adults, albeit this was deemed not to be statistically significant. The authors stated that their analysis did not provide sufficient evidence to propose different protein requirements for younger and older adults, but also noted their analyses were statistically underpowered to detect such a difference[79]. Campbell *et al.*[87] sought to provide additional data for older adults and found, similarly, no statistical difference between protein requirements for older and younger adults as well as between their calculated RDA (0·85 g/kg/d) and the current RDA[87]. In contrast, analysis of twenty-eight nitrogen balance studies with a non-linear regression analysis showed that the RDA was underestimating protein requirements for healthy adults; specifically, the EAR and RDA were reported to be 0·91 and 0·99 g/kg/d, respectively, which are estimates that are about 30 % greater than current requirements[81]. Researchers justified a non-linear biphasic, rather than a linear, regression model because: (1) the physiologic relationship between nitrogen intake and nitrogen balance is not linear, and (2) overestimated (higher protein intake) nitrogen balance data are less likely to significantly impact protein requirement calculations[81]. Also, the nitrogen balance technique has been criticised because it may present several limitations when determining protein requirements[83,88,89], creating a demand for the development of new and more accurate measurement techniques.

The indicator amino acid oxidation (IAAO) technique originated in animal nutrition but has since been utilised to determine protein requirements in human subjects[81,89–93]. The IAAO technique is predicated on the fact that ingested amino acids have no significant storage pool and must either be incorporated into tissues, metabolised or oxidised as fuel[94]. Thus, when a labelled amino acid is steadily ingested, a large proportion of the labelled amino acid will be oxidised and detectable in expired CO_2. As the amount of labelled amino acid incorporated into tissue increases, the oxidation detectable in expired CO_2 decreases. The observed oxidation will stop decreasing and plateau when the ingested amino acid has been maximally incorporated into tissue. Provided no other amino acids were limiting, biphasic linear regression performed on the oxidation data will yield a 'breakpoint', at which oxidation plateaus, representing the EAR for the ingested amino acid[93,94]. Compared to the nitrogen balance technique, the IAAO technique is favoured for determining amino acid requirements because it requires a shorter adaption time, about 2 d (*v.* about 10 d)[95], and is minimally invasive, thus lending itself nicely to vulnerable populations, such as older adults[93]. Using the IAAO technique in older men and women, Rafii *et al.*[96,97] calculated the EAR to be 0·94 and 0·96 g/kg/d and the RDA to be 1·24 and 1·29 g/kg/d, respectively[96,97]. Recently, Mao *et al.* utilised the IAAO technique in older Chinese adults and found the EAR and RDA for protein to be 0·91 and 1·17 g/kg/d, respectively[98]. Protein requirements calculated using the IAAO technique, though varied, are undoubtedly higher than the current EAR and RDA. Interestingly, a paucity of data prevented the IAAO technique from being utilised when determining the current protein requirements[80], so increasing data from IAAO studies may permit the technique to be utilised when updating the EAR and RDA.

Current measurements: considering skeletal muscle protein balance

Stable isotope tracer methods have permitted protein metabolism to be studied within skeletal muscle *in vivo*[63]. Similar to whole-body measures, several researchers have indicated that muscle protein metabolism differs between younger and older adults[99,100], such that protein requirements are likely to exceed the RDA for older persons. In a retrospective analysis, van Loon's group showed that postprandial MPS is significantly lower in older adults compared to younger adults following the ingestion of 20 g casein protein[17]. Importantly, sensitising skeletal muscle in older adults, such as with RE, is imperative to improve skeletal muscle anabolism, since older adults appear less responsive to smaller doses of dietary protein. Furthermore, the dose–response between protein ingestion and MPS is a saturable process[101], which, as Moore *et al.* demonstrated, requires larger per meal doses of protein in older adults (0·4 g protein/kg/meal) than younger adults (0·24 g protein/kg/meal) to maximally stimulate MPS[18]. Compared to younger adults, older adults retain the capacity to similarly stimulate MPS when provided a larger dose of dietary protein. Overall, understanding protein requirements within skeletal muscle is crucial to effectively treating sarcopoenia[63].

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Optimising protein nutrition

Total daily protein intake is an essential component of optimal nutrition, and several researchers have suggested that older adults need to increase their daily protein consumption. In a 10-week trial, older men were fed isoenergetic diets containing the RDA (0·8 g/kg/d) or twice the RDA (1·7 g/kg/d) of protein[102]. The authors showed that the higher protein group significantly increased lean body mass, particularly in the trunk region, and that consumption of the higher protein diet was accompanied by an increase in knee extension peak power[102]. Interestingly, a recent 12-week trial investigated the impact of a high-protein diet (1·4 g/kg/d) containing three eggs daily compared with a standard protein diet (0·8 g/kg/d) devoid of eggs[103]. The authors showed that the higher protein diet promoted greater lean mass retention, particularly in the trunk region, and had no pronounced negative impacts on cardiometabolic health or systemic inflammation[103]. The lack of adverse effects is appreciable since many older adults often avoid eggs because of perceived negative health implications[104]. In another 10-week trial, researchers conducted metabolomic analysis on older men's circulating plasma after consuming the RDA or twice the RDA of protein[105]. Notably, the high-protein diet increased markers of protein anabolism without any negative effects on pathways involved with metabolic health[105]. In addition to demonstrating the benefits of increased protein intake, utilising omic techniques represent a blossoming strategy in muscle physiology that should certainly be incorporated in future research. In a recent meta-analysis, researchers compared protein intakes greater than the RDA to protein intakes equal to the RDA in adults[106]. The authors demonstrated that consuming protein above the RDA (about 1·3 g/kg/d) favourably affected changes in lean mass, regardless of energy restriction or resistance training programmes[106]. This evidence aligns well with current recommendations for adults to consume 1·2–1·6 g/kg/d of protein[38], and suggests that increasing protein consumption promotes lean mass accrual, which could help mitigate future sarcopoenic declines. Altogether, increased daily protein intake seems beneficial for older adults' skeletal muscle health.

Despite an abundance of evidence for older adults to consume protein above the RDA, some researchers have produced contradictory findings. In a 10-week trial researchers compared the effect of high-protein (1·2 g/kg/d) *v.* standard protein (0·8 g/kg/d) diets with accompanying resistance training in postmenopausal women[107]. In the first trial, the researchers reported that both groups similarly increased lean mass[107]. Although this finding is, initially, puzzling, it suggests that RE is beneficial regardless of protein intake and that older women may need to consume more than 1·2 g/kg/d to result in lean mass accretion. Future trials investigating healthy ageing should incorporate higher protein intakes (e.g. 1·6 g/kg/d) with concomitant RE. In the second trial, the authors reported that higher protein intake improved functional capacity, as measured by the participants' performance on the Short Physical Performance Battery and various walking tests, but did not improve strength or lean mass[108]. Despite being somewhat inconsistent, these findings still show that consuming more protein and completing RE can yield beneficial results. Specifically, consuming more than 1·2 g/kg/d of protein while completing concurrent RE should be further tested.

A larger dose of dietary protein is required to produce a 'youthful' MPS response in older adults, as discussed earlier. Specifically, Moore *et al.* demonstrated that older adults need 0·4 g/kg protein per eating occasion, 67 % more than younger adults, to fully saturate MPS[18]. In a recent trial, Holwerda *et al.* assessed the effect of increasing doses of milk protein concentrate on MyoPS following RE in older men, and demonstrated that 30 or more g (≥about 0·37 g/kg) protein were required to maximally stimulate MyoPS[109]. These results align well with previous studies suggesting that older adults should consume 30–45 g protein per meal to optimise maintenance of muscle[110]. Researchers have also shown, albeit in younger adults, that ingesting food-based NDP sources may further potentiate MPS compared to isolated protein sources[22,74] speculating that non-protein components (vitamins, minerals, fatty acids, etc.) of the food matrices may further potentiate skeletal muscle anabolism via increased mechanistic target of rapamycin complex 1 signalling[73]. In addition to increased muscle anabolism, these high-quality protein sources represent an efficient method for older adults to improve diet quality and consume other nutrients pertinent to healthy ageing[111]. Older adults should prioritise nutrient-dense foods to maximise nutritional adequacy as they may represent a viable dietary option to combat sarcopoenia and promote healthy ageing.

Geriatric obesity and its confluence with sarcopoenia, leading to sarcopoenic obesity, is an increasingly prevalent health concern. In a 2016 meta-analysis, Kim *et al.*[112] assessed the impact of protein intake on changes in body composition amidst energy-restriction diets. Overall, their analyses demonstrated that older adults under energy restriction retained lean mass, while losing fat mass, when consuming ≥25 % total daily energy or ≥1·0 g/kg/d compared to older adults below either threshold[112]. Subsequently, a 16-week trial had obese older adults with metabolic syndrome consume a high-protein diet (1·4 g/kg/d) with or without concurrent RE training[113]. The authors reported that the high-protein, energy-restricted diet improved the health profile (e.g. total cholesterol and fasting glucose) of these individuals; however, RE was necessary to maintain lean body mass[113]. In a crossover trial, researchers utilised stable isotope tracer techniques to compare the effects of a bariatric beverage or IAA-enriched meal replacement on overweight older adults[114]. The IAA-enriched meal replacement resulted in significantly higher rates of protein synthesis and significantly lower rates of protein breakdown[114], highlighting the potential role of high-quality protein sources in weight-loss interventions. Together, researchers continue to demonstrate that consuming more high-quality protein with

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concomitant RE promotes a beneficial pattern of weight loss, less lean mass loss, in ageing populations.

Observational data

Recent observational data show older adults' protein nutrition needs to be improved. In an analysis of National Health and Nutrition Examination Survey (NHANES) 2005–2014 data (*n* 11,680, 50+ years old), Krok-Schoen *et al.* showed that 45 % of adults do not consume the current RDA for protein[115]. Individuals consuming less than the RDA for protein were also more likely to be below the EAR for zinc, vitamin C, vitamin D and vitamin E so it may be that these people were undernourished in general[115]. Researchers have also shown that protein consumption declines significantly throughout adulthood[116,117], and this observation held true when adults over 50 years old were compared by age[115]. Perhaps unsurprisingly, recent data show that older adults' per meal protein intake is largely inadequate compared to current recommendations. For example, another analysis of NHANES data showed that 71·4 % of older adults do not consume 25 g protein in at least one meal daily[118]. Additionally, with data from Ireland's National Adult Nutrition Survey (*n* 1051), Hone *et al.* showed that older adults reach the 0·4 g/kg of protein threshold[18], on average, less than once daily[116].

Several physiological benefits have been associated with protein consumption above the RDA. Krok-Schoen *et al.*[115] also demonstrated that older adults consuming less than the RDA were more likely to experience physical limitations, such as when kneeling, climbing stairs and walking 0·25 miles. Moreover, researchers found that older participants in the Health ABC study were significantly more likely to develop mobility limitations if they consumed less than 1·0 g/kg/d of protein[119]. Additionally, results from the Framingham Offspring Study showed that older adults consuming more than 1·2 g/kg/d, compared to those consuming less than the RDA, were 41 % less likely to become dependent for various functional tasks and had improved functional capacity; specifically, when lifting heavy items, walking 0·5 miles, and climbing stairs[120]. Importantly, the authors also showed that the combination of increased protein consumption and physical activity further reduced declines in physical function[120]. Interestingly, Landi *et al.*[121] observed that consuming animal-based protein was positively associated with muscle mass and strength, and that regular physical activity augmented their indices of muscle mass and function. Together, observational data indicate that consuming more protein, particularly from animal sources, positively contributes to skeletal muscle health in ageing.

Beyond total daily intake, observational studies have provided some evidence for the efficacy of protein dose and distribution. In an analysis of NHANES data, researchers showed that the frequency of consuming 30–45 g protein per meal, which typically equates to 0·4 g/kg/meal threshold, is positively associated with leg lean mass and strength[110]. Similarly, another group of researchers showed that higher protein intake at lunch, which seldom contains enough protein, was positively associated with muscle retention over 2 years[122]. In an analysis of NHANES data, researchers showed that even increasing the number of daily meals containing more than 0·25 g/kg of protein was associated with decreased odds of developing functional disability[118]. With respect to protein distribution, NuAge study researchers observed that a more level (i.e. even) distribution of protein intake was positively associated with higher muscle mass[123] and muscle strength[124] in older adults. Interestingly, researchers conducted a cross-sectional study and found that frail older adults had a significantly more skewed pattern of protein consumption compared to pre-frail and non-frail older adults[125]. Based on these data, consuming enough protein at each meal, perhaps via a more even distribution, appears beneficial for skeletal muscle health in older adults.

Despite the abundance of studies demonstrating that increased protein consumption is beneficial, some researchers have produced contrasting findings. For example, researchers investigated if total protein and type of protein, animal or plant, was related to changes in mid-thigh cross-sectional area in older adults over 5 years[126]. The researchers reported that there was no association between mid-thigh cross-sectional area and the amount of protein nor the type of protein consumed[126]; however, the authors discussed several potential reasons for these confounding results: (1) dietary protein was only measured once (year 2 of 5), so impactful dietary changes may not have been captured, (2) exercise was measured with self-reports, which may result in measurement error and (3) the computed tomography scan used to measure mid-thigh cross-sectional area, though precise, only captures a small portion of body composition[126]. Although difficult in studies of this magnitude, a more comprehensive array of measurements for physical activity and skeletal muscle may help elucidate the reasons for these results. Additionally, researchers recently reported that the relationship between daily protein intake and frailty status is dependent on the method used to assess frailty[127]. Moving forward, researchers should be mindful of potential differences when selecting assessment tools to measure various health indices, such as frailty. Overall, some researchers have produced somewhat confounding findings; however, the overwhelming portion of evidence suggests older adults should consume more dietary protein.

Potential negative outcomes of high-protein intake

Consuming NDP clearly contributes to healthy ageing; however, despite the observed benefits of consuming higher protein doses, the hypothesis that high-protein diets negatively affect kidney function is frequently raised[128]. Mechanistically, it is claimed that the increased renal solute load (i.e. urea) accompanying a high-protein diet leads to glomerular hyperfiltration[129],

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which would eventually lead to glomerular damage and declines in renal filtration[129]. A meta-analysis supported that a higher protein diet was associated with increased glomerular filtration rate (GFR)[130]; however, this meta-analysis only analysed GFR post-intervention, rather than the change in GFR following dietary interventions. As a result, such an analysis is unable to account for the net change in GFR over the nutritional interventions. Our group conducted a meta-analysis comparing GFR changes from baseline to post-intervention in trials where adult subjects consumed high-protein diets[128]. Unlike the conclusions using only post-intervention GFR[129,130], we found no difference in the GFR changes between a higher protein diet (≥1·5 g/kg or ≥20 % energy intake or ≥100 g protein/d) and normal/low protein diet (≥5 % less energy intake from protein/d compared high-protein diet)[128]. Notably, there was a trivial inverse correlation between the increase in GFR and ingested protein dose when examined using post-intervention only[128]. Importantly, the increase in GFR in response to increased protein ingestion (i.e. nitrogen load) is the normal adaptation of the kidney to increase solute clearance[131,132]. We acknowledge that increased GFR may be a process marker of kidney disease[133], but there is no evidence linking a higher protein diet to kidney disease in healthy individuals[131], and numerous studies have reported increased GFR after a high-protein diet[134–137].

Another debated negative outcome high-protein diets is impaired insulin action, which leads to insulin resistance and insulin-induced hypoglycaemia[138]. This hypothesis is based on the premise that the excessive insulin secretion and glucagon following high doses of protein disrupt the balance of the glucose production and disposal system[139,140]. Although a few previous studies performing acute ingestion of protein or amino acid infusion showed induction of insulin resistance by prevention of the insulin-mediated glucose disposal[141–143], no study has determined the chronic effects of higher protein diet on insulin actions and blood glucose concentration.

Additionally, a few studies have shown that protein restriction is associated with a longer lifespan[144,145]. Critically, most studies examining the relationship between protein restriction and lifespan used rodent[144] or drosophila[145,146] models. In a study that examined this relationship in human subjects, results were largely inconsistent depending on age; specifically, high-protein ingestion reduced mortality in those over age 65 years during an 18-year follow-up period, but increased overall mortality in those aged 50–65 years, although plant-based protein ingestion attenuated this increased mortality[147].

Based on available evidence, we recommend that older adults: (1) prioritise high-quality, nutrient-dense sources of protein (e.g. eggs, milk, beef, fish, diverse plant sources or leucine-enriched supplements); (2) Consume more protein spread throughout the day; specifically, increase the frequency of meals containing at least 0·4 g/kg of protein; (3) Engage in regular weight-bearing RE to amplify to the response to protein-containing meals.

Conclusion

Sarcopoenia is a disease that significantly impairs healthy ageing. Older adults should prioritise high-quality, nutrient-dense sources of dietary protein to facilitate maintenance of skeletal muscle and overall health. Animal-based protein sources, such as beef, dairy and eggs, are clearly beneficial for older adults. Consuming plant-based protein, though appealing, requires additional dietary planning to ensure adequate nutrition; moreover, a paucity of strong evidence prevents advocation for diets based exclusively on plant-based proteins. Furthermore, the current RDA continues to appear to be inadequate for older adults. Ageing adults can expect to experience several metabolic and functional benefits from consuming protein above the current RDA; specifically, older adults should aim to consume more protein each day (about 1·6 g/kg/d) and at each eating occasion (0·4 g/kg/meal), particularly at breakfast- and lunch-time meals. Provided the existing and emerging research demonstrating the benefits of improved protein nutrition, older adults may soon be taking a page out of Dickens' Oliver Twist and when it comes to high-quality NDP asking if they can have more?

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Conflict of Interest

S. M. P. reports that he is an inventor on a patent (WO/2018/157258) held by Exerkine Corporation and holds equity in Exerkine (no financial gains and all proceeds to charity). S. M. P. is a member, and receives an honorarium, of the scientific advisory board of Enhanced Recovery™ (all proceeds are donated to charity). S. M. P. has received, in the past 5 years, honoraria and travel expenses from the US National Dairy Council and the US Dairy Export Council.

Authorship

The authors had joint responsibility for all aspects of preparation of this paper.

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