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Nutrition strategies to counteract sarcopenia: a focus on protein, LC *n*-3 PUFA and precision nutrition

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Diminished skeletal muscle strength and size, termed sarcopenia, contributes substantially to physical disability, falls, dependence and reduced quality of life among older people. Physical activity and nutrition are the cornerstones of sarcopenia prevention and treatment. The optimal daily protein intake required to preserve muscle mass and function among older adults is a topic of intense scientific debate. Older adults require protein intakes about 67 % higher than their younger counterparts to maximally stimulate postprandial muscle protein synthesis rates. In addition, evidence suggests a possible benefit of increasing protein intake above the population reference intake (0·83 g/kg/d) on lean mass and, when combined with exercise training, muscle strength. In addition to protein quantity, protein quality, the pattern of protein intake over the day and specific amino acids (i.e. leucine) represent key considerations. Long-chain *n*-3 PUFA (LC *n*-3 PUFA) supplementation has been shown to enhance muscle protein synthesis rates, increase muscle mass and function and augment adaptations to resistance training in older adults. Yet, these effects are not consistent across all studies. Emerging evidence indicates that an older person's dietary, phenotypic and behavioural characteristics may modulate the efficacy of protein and LC *n*-3 PUFA interventions for promoting improvements in muscle mass and function, highlighting the potential inadequacy of a 'one-size-fits-all' approach. The application of personalised or precision nutrition to sarcopenia represents an exciting and highly novel field of research with the potential to help resolve inconsistencies in the literature and improve the efficacy of dietary interventions for sarcopenia.

Skeletal muscle: Leucine: Omega-3 fatty acids: Precision nutrition: Ageing

Abbreviations: EAA, essential amino acid; LC *n*-3 PUFA, long-chain *n*-3 PUFA; MPB, muscle protein breakdown; MPS, muscle protein synthesis; PRI, population reference intake; RCT, randomised controlled trials.

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Skeletal muscle is fundamental to mobility, health and physical independence. Beginning around the fifth decade of life[1], skeletal muscle mass and strength start to decline at a rate of about 0·8 % and about 2–3 % per year[2], respectively. Over time, these declines can lead to sarcopenia, a disease characterised by diminished skeletal muscle strength and muscle mass[3]. Sarcopenia increases risk of falls, fractures[4,5], functional decline[6] and physical disability[7]; it is a major component of physical frailty, and contributes to the loss of independence[8], the need for long-term care[9] and reduced quality of life[10]. The causes of sarcopenia include the ageing process *per se*, disease, physical inactivity and poor nutrition[3]. The underlying mechanisms are multifactorial and interrelated and may include, but are not limited to, neuromuscular deterioration[11], changes in endocrine function[12], vascular dysfunction[13], oxidative stress[14], low-grade chronic inflammation[15] and disruptions in muscle protein turnover[16,17].

As a result of population ageing, the total number of people affected by sarcopenia is expected to grow exponentially in the coming decades, with a projected rise from 10·9 million people in 2016 to 18·7 million people by 2045 in the European Union alone[18]. As such, identifying and implementing effective countermeasures to prevent and treat sarcopenia is imperative to support older adults in living, not only longer, but healthier and more independent lives. Physical activity and nutrition interventions are the first-line prevention and treatment strategies for sarcopenia, and there are currently no approved pharmaceutical interventions[19]. Physical activity, and in particular resistance exercise, is well established as the most effective strategy to counteract sarcopenia. Resistance exercise refers to physical activity which produces skeletal muscle contraction(s) by using external resistance such as free weights, resistance bands and body weight itself. Resistance training increases muscle mass[20], strength[21] and physical performance[22], and reduces disability risk[23] among older adults. Furthermore, it is effective in the oldest old[24], in those with pre-existing sarcopenia[25] and among those living in residential care[26].

Nutrition can also influence muscle mass, strength and physical performance in older adults[27], albeit to a lesser extent than exercise. Moreover, nutrition can augment resistance training-induced improvements in muscle mass and function[28,29], indicating that combined nutrition and physical activity strategies may be particularly effective for combatting sarcopenia. There are numerous nutritional components that may be relevant to preventing and treating sarcopenia (e.g. protein, energy, long-chain *n*-3 PUFA (LC *n*-3 PUFA), creatine, vitamin D, anti-oxidants, etc.). This review will specifically focus on the established and emerging roles of dietary protein and LC *n*-3 PUFA, respectively, with an emphasis on recent developments in personalised nutrition approaches for sarcopenia.

Muscle protein turnover in the regulation of muscle mass and quality

Skeletal muscle is a highly plastic tissue. The size and composition of skeletal muscle mass is determined, at least in part, by the constant turnover of skeletal muscle protein through the processes of muscle protein synthesis (MPS) and muscle protein breakdown (MPB)[30]. These processes function to degrade old and damaged proteins (MPB) and synthesise new proteins (MPS), thus impacting both skeletal muscle mass and quality[31].

In vivo rates of MPS and MPB can be measured using stable isotopic tracer techniques. Typically, this involves the intravenous infusion of an isotopically labelled (tracer) amino acid into the body combined with the collection of skeletal muscle biopsies[32]. During MPS, the tracer amino acid is taken up by the skeletal muscle and incorporated into new muscle protein. Thus, the rate of MPS can be determined by measuring the change in the muscle protein-bound enrichment of the tracer over a given period of time (using serial skeletal muscle biopsy samples) and the enrichment in the precursor pool (i.e. muscle intracellular free amino acids, plasma free amino acids, or ideally aminoacyl-tRNA)[32]. This labelled amino acid infusion technique provides sensitive measurements of MPS, and under controlled laboratory conditions, is ideal for assessing the acute response (about 2–24 h) to specific stimuli such as feeding or exercise. Unless otherwise stated, the studies included in this review have used the intravenous infusion of labelled amino acids to measure MPS rates. More recently, the oral consumption of deuterated water (^2H_2O), which leads to the endogenous ^2H-labelling of amino acids, has been used as an alternative to the infusion of pre-labelled amino acids. This method can be used to measure integrated MPS responses over longer time periods (days to weeks) in free-living settings. Compared to MPS, measuring MPB is substantially more challenging. Nonetheless, dynamic MPB rates can be determined in several ways, such as by the intravenous infusion of an isotopically labelled amino acid into the body and measuring the dilution of the tracer amino acid in the muscle intracellular pool (which occurs due to the appearance of unlabelled amino acids from MPB) or by using arteriovenous balance techniques[33].

Studies using stable isotopically labelled amino acid tracer infusion techniques have provided most of our contemporary understanding of the day-to-day regulation of skeletal muscle mass. The balance between MPS and MPB oscillates over the day in response to anabolic stimuli (i.e. dietary protein ingestion, muscle contraction). In the rested, fasted state, the rate of MPS is lower than that of MPB resulting in a negative net muscle protein balance (MPS < MPB) and muscle protein loss[34] (Fig. 1). The ingestion of dietary protein results in an increase in plasma essential amino acid (EAA) concentrations that stimulates a transient rise in MPS rates and, predominantly via the accompanying increase in plasma insulin concentration, suppresses MPB rates to produce a positive net muscle protein balance (MPS > MPB) and muscle protein accretion[30]. While both MPS and MPB are relevant to net muscle protein balance, it is the MPS arm that is the principal driver of the anabolic shift towards a positive net protein balance, with a comparatively smaller contribution from the reduction in MPB under normal physiological

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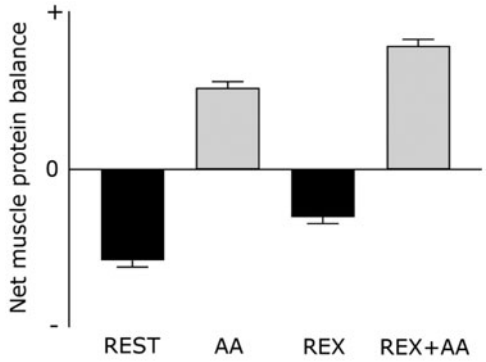

Fig. 1. Net muscle protein balance in the fasted, rested state (Rest), following amino acid ingestion (AA), after the performance of a session of fasted, resistance exercise (REX) and following a session of resistance exercise combined with post-exercise amino acid ingestion (REX + AA)[34,35].

conditions[35,36]. In healthy younger people, the consumption of protein-containing meals over the day results in approximately equivalent periods of negative (fasting) and positive (postprandial) net muscle protein balance on a day-to-day basis, thereby serving to maintain a stable muscle mass[30].

Besides protein consumption, exercise (muscle contraction) represents the other main anabolic stimulus for skeletal muscle. In the fasted state, the performance of a session of resistance exercise increases the rate of MPS and, to a lesser extent, MPB[34]. Under these conditions, there is a less negative, but still not positive, net protein balance[34] (Fig. 1). However, when dietary protein is consumed post-exercise, there is a synergistic effect on the stimulation of MPS and the ingested amino acids are used to synthesise new muscle protein[35,37]. This results in a protracted state of positive net muscle protein balance, which if repeated over time (i.e. via regular resistance training combined with adequate protein intake), leads to gradual muscle growth (hypertrophy), especially within the myofibrillar (i.e. contractile) protein fraction[38].

Disruptions in muscle protein turnover in ageing

Ageing is associated with disturbances in muscle protein turnover that favour a negative net muscle protein balance (MPS < MPB)[17,39]. The available data in humans suggest that in normal (non-pathological) ageing, this imbalance is primarily driven by a diminished MPS response to the normally robust anabolic stimuli of protein ingestion[16,39] and resistance exercise[17]. This phenomenon is termed 'anabolic resistance'. The mechanisms underlying anabolic resistance are not fully understood and a multitude of factors likely contribute. As exercise is known to sensitise the muscle to protein intake[37], while physical inactivity has the opposite effect[40], anabolic resistance is likely in part due to the lower physical activity levels among older adults compared with their younger counterparts[41]. In addition, impairments in protein digestion and amino acid absorption kinetics[42], insulin-mediated muscle tissue perfusion[43], muscle amino acid uptake[44] and a reduced amount or degree of activation of key signalling proteins involved in the muscle protein translational machinery[16] have all been reported in older adults and could result in reduced utilisation of ingested protein for MPS. Given that anabolic resistance is recognised to play a central role in age-related muscle loss, nutrition strategies targeted at sarcopenia prevention and treatment often aim to do so by overcoming anabolic resistance and enhancing MPS rates.

Dietary protein

Daily protein requirements to counteract sarcopenia

Dietary protein intake is well established as a key regulator of skeletal muscle anabolism[30]. Nonetheless, the optimal dietary protein intake required to preserve skeletal muscle mass and function among older adults is currently unclear and controversial. The population reference intake (PRI) of protein for the healthy adult population is set at 0·83 g/kg body mass/day by the WHO[45] and the European Food Safety Authority[46]. A similar recommended daily allowance of 0·8 g/kg/d is set by the Institute of Medicine in the USA[47]. These values are derived from a meta-analysis of nitrogen balance studies[48] and represent the estimated protein intake required to replace daily protein losses and prevent deficiency in 97·5 % of the population. Importantly, these values are recommended for all healthy adults regardless of age[45–47]. However, the appropriateness of these recommendations for older adults has been challenged[49–51]. It has been argued that the minimum amount of protein required to prevent deficiency (and maintain nitrogen balance) is unlikely to represent the quantity of protein required to optimise the maintenance of muscle mass and function among older adults who are at risk of sarcopenia[51]. Furthermore, of the nineteen studies included in the meta-analysis used to determine protein requirements[48], only one study[52] was conducted in older adults. Interestingly, the average nitrogen requirement was higher in the older adults (>67 years old; *n* 14) compared with a subgroup of younger adults (<40 years old; *n* 221) included in the meta-analysis, however the difference was not statistically significant[48].

Studies using isotopic tracers have repeatedly shown that older adults require more protein per meal to maximally stimulate MPS compared to younger adults at rest[39,53]. For example, a retrospective pooled analysis of six studies showed that whilst 0·24 g protein per kg body mass provided within a single dose was sufficient to maximise postprandial MPS rates in younger men, it resulted a diminished MPS response in healthy older men[39]. Nonetheless, at a higher dose of 0·4 g/kg the older men achieved similar maximal MPS rates to those of the younger men, suggesting that older adults can overcome the anabolic resistance to protein ingestion when higher quantities of protein are consumed[39].

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Further corroborating these data, rates of MPS measured over a 24 h period were higher when older men and women consumed diets providing 1·5 g protein/kg/d compared to 0·8 g/kg/d[54].

Several international groups of scientific experts advocate for a higher PRI for older adults than for younger adults to preserve muscle mass and potential function[49,50,55]. Specifically, they recommend daily intakes of 1·0–1·2 g/kg/d for healthy older adults[49,50]. As protein requirements may be further increased during illness (e.g. due to inflammation, infections, etc.), intakes of 1·2–1·5 g/kg/d have been recommended for older people with acute or chronic illness[49,50] and up to 2·0 g/kg/d in cases of severe illness, injury or malnutrition[49]. However, older adults with severe kidney disease (i.e. estimated glomerular filtration rate <30 ml/min/1·73 m^2) who are not on dialysis are an exception to the recommendations for higher protein intakes[49].

Recently, as part of a review of the PRI for protein for older adults in the Netherlands, the expert committee conducted a systematic review to examine the effect of increasing protein intake above the PRI on health outcomes in older people from the general population[56]. Based on the systematic review, which included eighteen randomised controlled trials (RCTs) and >1300 participants, the committee concluded that increasing protein intake above the current PRI (>0·83 g/kg/d) had a possible beneficial effect on lean body mass and, when combined with physical exercise (which was mostly resistance exercise), a possible beneficial effect on muscle strength[56]. While these effects on lean mass and strength may have importance, there was considerable inconsistency in the results of the evaluated studies. Moreover, the effects of higher protein intakes on lean mass and strength did not appear to translate into better physical performance. Thus, taken together, the committee judged that the existing RCTs did not provide sufficiently convincing evidence to warrant increasing the PRI for protein in healthy older adults beyond the current level (i.e. 0·83 g/kg/d)[56]. It is important to note that all the RTCs evaluated in the systematic review were judged to have either high or some risk of bias, due to issues such as lack of information on blinding, randomisation and missing outcome data[56]. Similar issues with poor study quality are apparent in the limited literature investigating the influence of differing protein intakes on muscle mass and function among older adults with sarcopenia, preventing clear recommendations for sarcopenia treatment[19]. Thus, further rigorously conducted studies are required to reach consensus on the optimal daily protein intake for the prevention and treatment of sarcopenia.

Protein source

Another consideration in relation to the prevention and treatment of sarcopenia is the quality of the protein in the diet. The 'quality' of a protein source is contingent on its EAA composition and digestibility[57], factors that are also critical determinants of a protein's capacity to stimulate MPS[58–60]. The stimulation of MPS following protein ingestion is primarily attributable to the increase in plasma EAA concentrations[61]. EAA serve not only as substrates for the synthesis of new muscle but can also act as signalling molecules that trigger the MPS response[62]. In general, animal-based proteins provide a better quality of protein compared to plant-based sources due to differences in EAA content and protein digestibility[47]. Proteins from animal sources such as meat, fish, eggs and milk-based products tend to have a higher total EAA content compared to most plant sources such as legumes, grains, nuts and seeds. Animal protein sources provide all nine of the EAA that are required as precursors for the synthesis of new muscle protein, whereas plant-based sources are often deficient in one or more of the EAA, particularly lysine or methionine[47,63]. Animal proteins also often have a higher content of leucine[64], an EAA known to play a key role in triggering the postprandial stimulation of MPS[62,65]. In addition to the differences in EAA content, the protein digestibility of plant-based whole foods is generally lower than animal-based foods, resulting in a smaller fraction of the amino acids ingested from plant proteins becoming available in plasma where they can serve as building blocks and signals for the synthesis of muscle. For example, recent data in human subjects show that about 85–95 % of the protein in eggs and chicken is absorbed[66], compared with only about 50–75 % of the protein in legumes[67]. This is less of an issue for isolated plant proteins where the anti-nutritional factors (e.g. fibre, protease inhibitors, tannins) that interfere with protein digestion and absorption are removed, typically resulting in protein digestibility that is equivalent to that of animal sources[68,69].

Few studies have directly compared the MPS response following the ingestion of a plant-derived protein *v.* a higher quality animal-derived protein in older adults. Beef[70] and whey protein[59] have been shown to stimulate postprandial MPS to a greater extent than an isonitrogenous amount of isolated soy-based protein in older adults, both at rest and following resistance exercise. Nevertheless, it may be possible to overcome the lesser anabolic properties of plant-derived proteins by consuming a larger amounts of protein. For example, Gorissen *et al.* showed that 60 g, but not 35 g, of isolated wheat protein hydrolysate increased MPS rates above fasting levels in older adults[71]. Although this is interesting from the perspective of understanding the regulation of MPS, consuming such a large quantity of protein from a single plant-based source is impractical for older adults. Plant-based protein sources generally have a lower protein density meaning that large food volumes and energy intakes would be required to achieve the target protein dose (e.g. 60 g of wheat protein is equivalent to about 16 slices of bread).

A more feasible strategy to increase the anabolic response to a plant-based protein is to combine it with different protein sources to provide a more 'complete' amino acid profile/a higher leucine content. Studies in younger[72] and older individuals[73] reported that the ingestion of a blend of soy and dairy proteins (whey and casein) stimulated MPS rates to a similar extent as

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an equivalent dose of whey protein following resistance exercise. This strategy is particularly relevant as, in the real world, mixed meals habitually consumed by older adults are often composed of a combination of plant and animal protein sources. It is currently unknown whether combining multiple plant-based protein sources to allow for the ingestion of a 'complete' EAA profile within a single plant-only meal can improve the post-prandial MPS response compared with the consumption of a single plant-based protein source, and this requires investigation.

To date, the majority of studies investigating the impact of various sources of protein on MPS have utilised isolated protein sources. However, the vast majority of protein consumed by older adults is in the form of whole foods or mixed meals. An emerging body of work suggests the food matrix may influence the MPS response[74,75]. As such, further work exploring the influence of whole foods and mixed meals on MPS rates will help inform dietary guidelines for older adults[76]. Furthermore, studies comparing the impact of isonitrogenous diets composed of different protein sources (e.g. plant *v.* animal) on longer-term changes on muscle mass and function in older adults are lacking. Such research will be important given the trends towards plant-based dietary patterns for environmental and sustainability reasons.

Leucine

Leucine is the most potent EAA in terms of its ability to stimulate MPS[77,78]. This effect is mediated, at least partially, via the activation of the mammalian target of rapamycin complex 1, resulting in the activation of the downstream anabolic signalling pathways that control MPS[78]. The leucine content of a protein source appears to play a central role directing the magnitude of the post-prandial MPS response, especially in older adults[79] (Table 1). Indeed, the greater leucine content of whey protein (about 11 %) is thought to largely explain the higher MPS response to whey ingestion relative to the same dose of soy protein (about 7 % leucine)[59,63]. Numerous amino acid tracer infusion studies in older adults have reported that the postprandial MPS response to a suboptimal dose of isolated protein (≤20 g) is enhanced by leucine fortification[80–82]. Furthermore, co-ingesting 5 g of crystalline leucine alongside each of the daily meals (breakfast, lunch, dinner) was shown to augment daily MPS rates alone and in combination with resistance exercise, in a study that used deuterated water to capture integrated MPS rates in older men under free-living conditions[83]. Moreover, in that study, leucine co-ingestion was effective regardless of whether the participants were consuming protein intakes at the PRI (0·8 g/kg/d) or above the PRI (1·2 g/kg/d)[83].

In longer-term studies, crystalline leucine supplementation (2·5 g co-ingested with the three daily meals) for 3–12 months did not promote improvements in lean mass, strength or physical performance in healthy older men[84], older men with type 2 diabetes[85] or older men and women with sarcopenia[86]. However, studies investigating the impact of combined leucine supplementation and resistance training are lacking. Conversely, leucine-enriched protein supplementation has been shown in some[87–89], but not all[90,91], studies to exert beneficial effects on lean mass and function in healthy and sarcopenic older adults, when provided alone[87,88] and in combination with exercise training[89]. For example, in a multicentre, randomised, double-blind controlled trial of 380 sarcopenic older men and women, 13 weeks of twice daily supplementation with leucine-enriched protein (3 g leucine, 20 g protein) plus vitamin D (20 µg) increased appendicular lean mass and five times sit-to-stand time (a measure of physical performance)[87]. Nonetheless, it is unclear from this trial whether the observed benefits were due to the leucine-enriched protein, the vitamin D, or their combination. Indeed, in several trials reporting improvements in muscle mass and/or function following

Table 1. Protein and leucine content of common foods

Food	Average portion (g)	Household measure	Protein (g)	Leucine (g)
Beef (roasted)	90	2 thick slices	33	2·6
Chicken (grilled)	100	Small chicken fillet	29	2·2
Tuna (canned)	80	½ standard tin	20	1·7
Sardines (canned)	85	3 sardines	20	1·7
Whole milk	250	1 glass	9	0·8
Chickpeas	120	½ standard tin	9	0·6
Eggs (cooked)	60	1 large	8	0·7
Kidney beans	120	½ standard tin	8	0·7
Bread (whole wheat)	74	2 average slices	7	0·5
Brown rice (cooked)	160	4 heaped Tbsp	6	0·5
Soy beverage	250	1 glass	6	0·5
White rice (cooked)	160	4 heaped Tbsp	5	0·4
Peas (cooked)	60	2 Tbsp	5	0·4
Oats (cooked)	30 (dry weight)	Medium bowl cooked porridge made with water	3	0·3
Maize-based breakfast cereal	45	Medium-large bowl	3	0·4
Potato (boiled)	180	2 small old potatoes	3	0·2

Average portion sizes based on the Irish food portion sizes database[131]. Protein and amino acid content of foods derived from Nutritics (2019) Research Edition (v5·09) using the GB15 database.

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supplementation with leucine-enriched protein in older adults, vitamin D_3 was co-ingested within the supplement[87–89]. *In vitro*, vitamin D_3 treatment was shown to sensitise muscle cells to the stimulatory effects of leucine and insulin on MPS[92] suggesting that synergistic effects may occur with combined supplementation. Thus, further work is needed to ascertain if, and under which conditions, leucine and/or leucine-enriched protein may be beneficial in sarcopenia prevention and treatment.

Pattern of daily protein intake

In addition to total daily protein intake, the amount of protein consumed per meal may also be important. The ingestion of dietary protein leads to a transient rise in the rate of MPS that returns to fasting levels after about 3 h[93,94]. There is a saturable, dose–response relationship between the amount of protein consumed in the meal and the subsequent MPS response[39]. Studies show that older adults require 0·4 g protein/kg/meal (equivalent to about 25–40 g protein/meal) to optimally stimulate postprandial MPS, under resting conditions[93]. A similar dose of ≥0·37 g/kg/d is required post-resistance exercise[95]. Thus, it is proposed that an even distribution of total protein intake, with the ingestion of at least 0·4 g/kg of protein at each meal, would effectively stimulate MPS over the day[96]. Nonetheless, older adults typically consume protein in a skewed pattern, eating a disproportionately high amount of the daily total protein intake at the main meal (about 40–50 %) and smaller, suboptimal amounts (about 0·1–0·3 g/kg) at the other meals[97,98]. As such, community-dwelling older adults report consuming ≥0·4 g protein/kg at only one meal daily, on average[98].

In isotopically labelled amino acid infusion studies measuring MPS over 12–24 h, an even distribution of protein was shown to stimulate MPS to a greater extent than an skewed pattern among older adults undergoing weight loss[99], as well as in younger adults at rest[100] and during recovery from resistance exercise[101]. However, several other studies have shown similar daily MPS rates in response to an even and a skewed pattern of protein intake in older adults using labelled amino acid infusion[54,102] and deuterated water[103] methods. A possible reason for the discrepancy may relate to the fact that protein was provided via whole foods within mixed meals in the studies that did not observe a favourable effect of an even protein intake pattern on MPS in older adults[54,102,103]. As the 0·4 g/kg/meal target protein dose is derived from studies that fed high-quality isolated protein[39], it is possible that a higher per meal protein dose is required when protein is consumed via a mixed meal to account for the varied protein quality and altered amino acid kinetics[104]. Supporting this notion, a positive association between per meal protein intake, leg lean mass and strength was observed among older adults at a level of 45 g protein/meal (or 0·55 g protein/kg/meal) in the National Health and Nutrition Examination Survey[105]. Nonetheless, further work is required to empirically test this hypothesis.

RCTs exploring the impact of an even distribution of optimally stimulatory per-meal doses of protein on muscle mass and function are limited. Kim *et al.*[102] observed no differences in lean mass, strength or other functional outcomes following 8 weeks of controlled diets providing protein in either an even (about 0·37 g/kg/meal) or skewed pattern, in healthy older men and women. However, as the effects of nutrition interventions on skeletal muscle are subtle, the short intervention duration and low sample size (*n* 19) of the study would likely have limited the ability of the investigators to detect an effect of the protein intake pattern, if it were present. Interestingly, Bouillanne *et al.*[106] reported that a 6-week skewed pattern of protein intake improved in lean mass compared to an even pattern (0·22–0·38 g/kg/meal) among hospitalised, malnourished or at-risk older adults. Importantly however, the quantity of protein required to maximally stimulate MPS in this group of older adults may be substantially higher than the 0·4 g/kg/meal dose previously reported for healthy older adults[39] due to energy deficit[99], inflammation[107] and low physical activity levels[40]. In this study, the large protein dose at the main meal (about 0·94 g/kg) in the skewed group was likely sufficient to maximally stimulate MPS, potentially leading to the observed improvement in lean mass[31]. Thus, it may be that different protein intake patterns/per-meal protein intakes are required for different individuals depending on the person's phenotype, activity levels, nutritional status and the food matrix in which the protein is consumed.

LC n-3 PUFA

EPA and DHA are LC *n*-3 PUFA consumed in the diet predominantly through seafood (especially oily fish) and supplements. Recently, evidence has started to accumulate to suggest that EPA and DHA may influence skeletal muscle mass and function. Several epidemiological studies have reported that the oily fish consumption is positively associated with muscle function in older populations[108,109]. Intervention studies show that LC *n*-3 PUFA supplementation increases the EPA and DHA content of skeletal muscle and alters the lipid composition of key regulatory sites, such as the sarcolemma and mitochondria[110,111].

Seminal work from Smith *et al.* demonstrated that fish oil supplementation (1·9 g/d EPA + 1·5 g/d DHA) for 8 weeks augmented the MPS response to an amino acid and insulin infusion in healthy, but sedentary, older adults; suggesting that LC *n*-3 PUFA intake may attenuate anabolic resistance[110]. This increase in MPS was accompanied by enhanced activation of several key molecules in the mammalian target of rapamycin complex 1 signalling axis, an integral pathway involved in the regulation of MPS[110]. In a follow-up double-blind RCT, the same investigators demonstrated that longer-term (24 weeks) supplementation with the same dose of EPA + DHA increased thigh muscle mass and upper- and lower-body muscle strength in forty-four healthy, sedentary older adults[112]. These findings that were recently corroborated by another research group using a slightly lower dose of 0·77 g/d EPA + 0·38 g/d DHA provided as a krill oil supplement[113]. Importantly, the observed treatment effects were clinically relevant, as

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the increase in isometric knee extensor maximal torque (9·3 % (95 % CI 2·8, 15·8 %)) exceeded a previously defined minimally clinically important difference of 3·7 %[113,114]. There is also some evidence that LC *n*-3 PUFA supplementation may enhance skeletal muscle adaptations to resistance exercise training in older adults[29,115]. In a double-blind RCT, Da Boit *et al.*[29] demonstrated improved strength gains in older women who were supplemented with LC *n*-3 PUFA (2·1 g/d EPA + 0·6 g/d DHA) compared to a placebo during 18 weeks of supervised resistance training. Intriguingly, this effect of LC *n*-3 PUFA was not apparent among the older men in the study, suggesting that a sex-based difference may exist in responsiveness to LC *n*-3 PUFA. However, further research is needed to confirm this.

Despite several trials reporting favourable effects of LC *n*-3 PUFA supplementation on skeletal muscle outcomes in older adults, not all studies have observed beneficial effects[90,116,117]. For example, in a recent double-blind RCT in 107 older adults with lower muscle mass, 24 weeks of LC *n*-3 PUFA fish oil supplementation (1·6 g/d EPA + 2·2 g/d DHA), provided as part of a mixed macronutrient drink containing leucine-enriched protein, did not favourably impact appendicular lean mass, strength or physical performance[90]. Similarly, 1 year of supplementation with 0·8–1·6 mg/d EPA + 0·4–0·8 mg/d DHA failed to improve 400 m walking speed in a study of 289 older adults with mobility impairment[117]. The DO-HEALTH trial is the largest study to date (*n* 2000) to examine the influence of LC *n*-3 PUFA supplementation on muscle health in older adults[116]. This study reported no influence of LC *n*-3 PUFA supplementation (0·3 g EPA/d + 0·7 g DHA/d) for 3 years, either alone or when combined with mild, twice-weekly homebased strength training, on the primary outcome of physical performance measured via the short physical performance battery (SPPB)[116]. Interestingly however, LC *n*-3 PUFA supplementation modestly reduced the incidence rate of falls by 10 %[118], an effect which may be related to favourable effects of LC *n*-3 PUFA on muscle function that were not detected by the SPPB test and/or due to extra-muscular effects (e.g. cardiovascular, cognitive).

The reason for the inconsistency in the literature regarding the impact of LC *n*-3 PUFA supplementation is unclear. Possible reasons for this inconsistency may include variations in the supplement dose or composition, intervention duration, the characteristics of the population studied (e.g. age, health status, physical activity levels, proportion of females, nutritional status, etc.) and the sample size. Yet, when comparing studies reporting favourable effects of LC *n*-3 PUFA supplementation to those observing no effect, no clear pattern emerges with respect to the aforementioned factors. For example, studies that have reported favourable effects of supplementation have provided EPA + DHA doses of 0·7–3·4 g/d[112,115] compared with a similar dose range of 1·0–3·8 g/d[90,116] in studies that have not observed beneficial effects. One potentially important consideration is the LC *n*-3 PUFA status of participants at baseline, as it is conceivable that participants with a lower status would be more likely to benefit from increased LC *n*-3 PUFA intake. Unfortunately, it is difficult to compare LC *n*-3 PUFA status across studies due to a variation in how LC *n*-3 PUFA status is reported, and no study has specifically recruited participants with low LC *n*-3 PUFA status at the outset. Finally, the outcomes measured may also account for some of the apparent inconsistency in the data. For example, in the DO-HEALTH study, the median baseline score for the primary outcome, SPPB score, was eleven out of twelve indicating that there was limited scope for improvement due to the intervention. DO-HEALTH have collected secondary (grip strength, incidence of decline in appendicular lean mass) and exploratory (incident frailty, incident sarcopenia) muscle-related endpoints which will presumably be reported in the future[116], and will likely make an important contribution to the literature regarding the utility of LC *n*-3 PUFA supplementation in sarcopenia prevention.

Personalised nutrition and sarcopenia

Interindividual variability in responses to nutrition interventions has been documented for decades[119–121] highlighting the potential limitations of a 'one-size-fits-all' approach. A range of hereditary and acquired characteristics, including a person's baseline phenotype, genotype, habitual diet, lifestyle behaviours and environment may alter the effect of a nutrition intervention, making it more or less effective in different individuals[122]. These observations have led to the field of personalised or precision nutrition whereby researchers aim to understand which individuals are more or less likely to respond favourably to specific nutrition interventions and why, with the ultimate goal of enhancing dietary intervention efficacy.

The application of personalised nutrition to sarcopenia is an exciting new field of research with the potential to improve outcomes in older individuals. Sarcopenia represents a condition that stands to benefit substantially from a more personalised perspective for a multitude of reasons[123]. Firstly, there are a host of different, yet interrelated, mechanisms involved in the development of sarcopenia (e.g. changes in hormones, oxidative stress, inflammation, alterations in blood flow, anabolic resistance, neural changes, etc.)[124]. As the relative contribution of each putative mechanism likely varies between individuals, distinct nutrition interventions, or combinations thereof, could be expected to be more efficacious in different individuals. Secondly, in sarcopenia, a positive dietary intervention outcome is represented by either the maintenance or a very slight increase in muscle mass and function. Importantly, any diet-induced changes occur very slowly, so prolonged intervention periods are required to establish therapeutic efficacy. As such, identifying biomarkers that can predict whether an individual is likely to be a higher or lower responder to a particular nutrition strategy could have important implications for clinical management of sarcopenia. Thirdly, as mean outcomes may mask higher and lower responder populations, interrogating the interindividual variability in responses may help to resolve

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inconsistencies in the literature regarding the impact of various nutritional strategies (e.g. protein, LC *n*-3 PUFA supplementation) on muscle mass and function.

In the precision nutrition field, data analysis approaches need to be very robust in terms of defining response *v.* non-response or higher- *v.* lower-response. Indeed many studies to date have not necessarily differentiated between biological noise *v.* a true change in response to the intervention[125,126]. Recently, we published the first study to characterise the interindividual variability in response to a nutrition intervention for sarcopenia[127]. In that study, older adults at risk of sarcopenia were supplemented with drinks containing either: leucine-enriched protein (LEU-PRO; 10 g protein, 3 g leucine), leucine-enriched protein plus fish oil (LEU-PRO + *n*-3; 0·8 g EPA, 1·1 g DHA) or an energy-matched control (CON), twice daily for 24 weeks[127]. At the mean group level, there were no beneficial effects of either LEU-PRO or LEU-PRO + *n*-3 supplementation on appendicular lean mass, strength and physical performance[127]. However, it was hypothesised that some individuals in the cohort may have responded to a greater degree than reflected by the group means, potentially due to individual characteristics such as their baseline protein intake, LC *n*-3 PUFA status or other phenotypic (e.g. sex, inflammatory status, vitamin D status) or behavioural (e.g. physical activity levels) characteristics. For example, it is logical that dietary protein supplementation may help preserve muscle mass among individuals with inadequate protein intake, but is unlikely to provide further benefit among those already consuming optimal quantities of protein. In the same way, older adults with poor LC *n*-3 PUFA status may be expected to benefit from LC *n*-3 PUFA supplementation to a greater extent than individuals with higher LC *n*-3 PUFA status. Nonetheless, counter to the hypothesis, after accounting for the effects of measurement error and within-subject variation, there was little evidence of clinically meaningful interindividual variation in the appendicular lean mass, strength and physical performance responses to either LEU-PRO or LEU-PRO + *n*-3 supplementation[127]. Moreover, no associations between baseline protein intake, LC *n*-3 PUFA status or any other measured phenotypic, dietary or behavioural variables and responsiveness to LEU-PRO or LEU-PRO + *n*-3 were observed[127]. Nonetheless, as this study was a secondary analysis of an RCT, it was powered based on the expected mean change in the primary outcome rather than for the analysis of individual responses. As such, larger studies are required to determine whether true interindividual variability exists in the responses of older adults to LEU-PRO or LEU-PRO + *n*-3 supplementation.

Several other studies have explored whether individual participant characteristics may modulate responsiveness to nutrition interventions for sarcopenia. Da Boit *et al.* reported that LC *n*-3 PUFA supplementation enhanced adaptations to resistance training in older women but not older men[29]. Further suggesting that women may be more responsive to the effects of LC *n*-3 PUFA supplementation, a sub-group analysis of the DO-HEALTH trial reported that the beneficial effects of LC *n*-3 PUFA supplementation on reducing falls incidence were more evident in older women than older men[118]. This study also reported enhanced responsiveness to LC *n*-3 PUFA supplementation among those who were older (i.e. aged ≥75 years as compared with those aged 70–74 years), were more physically active (≥26·3 MET-hr/wk) and had higher plasma LC *n*-3 PUFA concentrations (≥100 µg/ml) at baseline[118]. The observation that participants with higher LC *n*-3 PUFA status at baseline had a greater reduction in falls incidence in response to LC *n*-3 PUFA supplementation is at odds with our reasoning above that older adults with lower LC *n*-3 PUFA status may be more likely to benefit from supplementation. Nonetheless, as the dose of LC *n*-3 PUFA provided in DO-HEALTH was quite low (0·4 g/d EPA + 0·7 g/d DHA), it may be that this dose was only capable of raising LC *n*-3 PUFA status to a threshold associated with falls reduction among participants with higher LC *n*-3 PUFA status at baseline. A similar phenomenon was also apparent in the PROVIDE study wherein sarcopenic older men and women who were vitamin D sufficient (≥50 nmol/L) and had higher protein intakes (≥1 g/kg/d) at baseline had greater increases in appendicular lean mass in response to 13 weeks of leucine-enriched protein plus vitamin supplementation compared to those with lower vitamin D status and protein intake at baseline[128]. Taken together, these data suggest that poorly nourished older adults may be less responsive to nutrition interventions for sarcopenia and/or that the optimal intakes of these nutrients may be even higher than is currently thought. Body composition is another characteristic that may modulate an individual's response to protein intake. Smeuninx *et al.*[129] reported that the MPS response to the ingestion of 15 g of milk protein was lower in older men with obesity compared to age-matched lean men. This suggests that high body fat levels may exacerbate anabolic resistance to protein intake and could potentially influence the responsiveness of an individual to longer-term protein supplementation and/or the dose required for efficacy.

In summary, existing research indicates that participants' dietary, phenotypic and behavioural characteristics may modulate the efficacy of dietary interventions targeted at improving skeletal muscle mass and function in older adults. However, considerably more work is needed in this space. Importantly these studies will need to be larger (e.g. approximately four times the sample size needed to observe a mean effect[130]) which represents a significant challenge. Nonetheless, this work will be imperative in helping to understand the interindividual variability in older adults' responses to different nutrition interventions for sarcopenia prevention and treatment, in order to enhance the efficacy of dietary interventions.

Future directions and conclusion

It is well accepted that adequate protein intake is important for sarcopenia prevention and treatment. Older adults require more protein (and leucine) than younger

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adults to optimally stimulate MPS. Nonetheless, the optimal daily protein intake required to preserve muscle mass and function in older adults is unclear and requires further investigation in rigorously controlled trials. Ingestion of plant-derived proteins (soy, wheat) have been reported to result in lower post-prandial MPS responses compared with the ingestion of equivalent amounts of animal-derived protein (milk proteins, beef) in older adults. However, future work is needed to compare a wider variety of plant- and animal-based protein sources, including whole foods and, most importantly, complete meals. This work will have important implications for refining guidelines for healthy and sustainable diets for older people. Emerging evidence suggests that LC *n*-3 PUFA supplementation may promote skeletal muscle anabolism and strength in older adults, however the findings of studies are not consistent. Existing research indicates that an individual's dietary, phenotypic and behavioural characteristics may modulate the efficacy of protein and LC *n*-3 PUFA interventions targeted at improving skeletal muscle mass and function in older adults. The application of personalised nutrition to sarcopenia represents an exciting and highly novel field of research with the potential to improve the efficacy of dietary interventions. Nevertheless, further research, coupled with robust methods to define individual nutrition intervention response, is required to establish the extent to which this approach is effective.

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Conflict of Interest

None.

Authorship

C. H. M. wrote the manuscript; S. N. McC. and H. M. R. read, revised and approved the manuscript; C. H. M. had primary responsibility for the final content.

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