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Dietary protein recommendations to support healthy muscle ageing in the 21st Century and beyond: considerations and future directions.

Invited review article (for the Proceedings of the Nutrition Society)

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Abstract

This review explores the evolution of dietary protein intake requirements and recommendations, with a focus on skeletal muscle remodeling to support healthy ageing based on presentations at the 2023 Nutrition Society summer conference. In this review, we describe the role of dietary protein for metabolic health and ageing muscle, explain the origins of protein and amino acid requirements, and discuss current recommendations for dietary protein intake, which currently sits at ~0.8g·kg^{-1}day^{-1}. We also critique existing (e.g., nitrogen balance) and contemporary (e.g., indicator amino acid oxidation) methods to determine protein/amino acid intake requirements and suggest that existing methods may underestimate requirements, with more contemporary assessments indicating protein recommendations may need to be increased to >1.0g·kg^{-1}day^{-1}. One example of evolution in dietary protein guidance is the transition from protein requirements to recommendations. Hence, we discuss the refinement of protein/amino acid requirements for skeletal muscle maintenance with advanced age beyond simply the dose (e.g., source, type, quality, timing, pattern, nutrient co-ingestion) and explore the efficacy and sustainability of alternative protein sources beyond animal-based proteins to facilitate skeletal muscle remodeling in older age. We conclude that, whilst a growing body of research has demonstrated that animal-free protein sources can effectively stimulate support muscle remodeling in a manner that is comparable to animal-based proteins, food systems need to sustainably provide a diversity of both plant and animal source foods, not least for their protein content but other vital nutrients. Finally, we propose some priority research directions for the field of protein nutrition and healthy ageing.

Introduction

The topic of protein nutrition is continually evolving with considerable interest in recommendations for skeletal muscle health across the health- and lifespan continuum. Proteins, or more specifically their constituent subunits of amino acids (AA), represent the building blocks of body tissues, including muscles (skeletal, cardiac and smooth), bone, skin, and organs. A large proportion of ingested dietary protein-derived AA are directed to these peripheral tissues following extraction by the splanchnic tissues (intestine, stomach, spleen, pancreas)(1–3). Dietary protein is essential for various physiological functions including movement (e.g., contractile proteins, tissue remodeling), structure (e.g., collagen), transport and storage (e.g., haemoglobin), cell signaling (e.g., communication pathways), enzymes (to facilitate biochemical reactions), immune function (e.g., antibodies), hormones as chemical messengers regulating various physiological processes (e.g., insulin) and receptors (e.g., insulin receptor), as well as energy provision. Hence, protein nutrition plays a crucial role in human health across the lifespan, as well as during recovery from catabolic stress (e.g., frailty, cancer cachexia, surgery, sepsis, enforced physical inactivity / disuse, energy restriction)(4–7). This brief synopsis of an oral presentation delivered at the 2023 UK Nutrition Society summer conference (Nutrition at key stages of the lifecycle) explores the evolution of dietary protein requirements and recommendations, with a focus on skeletal muscle remodeling to support healthy ageing. The main purpose of this review is to (i) discuss how dietary protein requirements (i.e., what is needed for survival) and recommendations (i.e., scientific guidelines to achieve optimal biological outcomes) have evolved in the context of healthy ageing and (ii) provide concise, evidence-based, and practically relevant protein guidelines for older adults with a focus on skeletal muscle health. For a recent critical narrative review of the scientific evidence on dietary protein requirements and recommendations for healthy older adults, see Nishimura *et al*., (2023)(8).

Musculoskeletal health in an ageing society: a role for dietary protein?

Globally, ageing is associated with increased healthcare costs and social service needs(9). In addition, the gap between lifespan (i.e., total lived age) and health span (i.e., years of life free from disease)(10,11) continues to grow, compounded by a decrease in habitual physical activity levels and increased prevalence of diseases associated with advanced age(12,13). Indeed, lifelong engagement in exercise (e.g., Master athletes) results in the better maintenance of skeletal muscle mass into older age and may be considered a more true model of inherent ageing (i.e., represents ageing, per se, rather than the detriments seen due to inactivity)(14,15). Moreover, while the cause(s) of age-related muscle loss (otherwise termed ‘sarcopenia’) is clearly multifaceted, a key contributor is malnutrition, and in particular a reduced dietary protein intake(16). Indeed, higher protein intakes have been associated with greater retention of lean mass in older individuals in some(17), but not all(18), studies. Hence, with advanced age, it seems prudent to tailor protein intake recommendations to counter age-related changes in the metabolic response of skeletal muscle to ingested protein, as well as reduced physical activity. Importantly, with regard to attenuating age-related muscle loss, the roles of skeletal muscle go beyond locomotion to critical actions such as chewing and swallowing, breathing, maintenance of body posture and thermogenesis. Combined with the misalignment of health- and lifespan, this highlights an urgent unmet need in an ageing society to comprehensively understand protein intake requirements and develop appropriate recommendations.

Skeletal muscle protein synthesis: a primary role of dietary protein

The primary nutritional value of dietary protein is the provision of AA for the synthesis of new, functional proteins, including skeletal muscle (termed muscle protein synthesis, MPS). While a sufficient quantity of non-essential amino acids (NEAA) can be supplied endogenously, an exogenous (e.g., dietary) supply of essential amino acids (EAA, sometimes referred to as ‘indispensable’ AA) is necessary for the stimulation of MPS, subsequent skeletal muscle remodeling and to remain in a positive (or net) protein balance(19). Indeed, all body tissues including skeletal muscle remain in a constant state of turnover, with the old, damaged proteins most likely degraded (via muscle protein breakdown) concurrently with the synthesis of new, functional proteins (via muscle protein synthesis)(3). Whilst muscle loading, via exercise/physical activity, represents the most potent stimulator of MPS and skeletal muscle remodeling(20), in the absence of a sufficient exogenous supply of all nine EAA, skeletal muscle will remain in a state of net negative protein balance (i.e., net protein synthesis < net protein breakdown) that will ultimately lead to muscle loss and the associated metabolic, morphological, and functional consequences(13). Moreover, dietary protein is required throughout life to replace irreversibly oxidized AA that cannot be synthesized in the body (i.e., EAA) and is particularly important given that protein is the only macronutrient that does not have an inactive compartment to serve as a reservoir. Accordingly, in practice, each of the >1000 meals consumed across a year, assuming 3 main meals/day, provides an opportunity for dietary protein to support skeletal muscle remodeling to attenuate the loss of skeletal muscle that is typically observed with advancing age(13).

A brief historical perspective on devised protein requirements and recommendations for adults

According to published records, proteins were first recognized as a distinct class of biological molecules by French chemist Antoine-François Fourcroy in the 18th Century and described by the Dutch chemist Gerardus Johannes Mulder as *"unquestionably the most important of all known substances in the organic kingdom. Without it, no life appears possible on our planet. Through its means, the chief phenomena of life are produced"* (21,22). *S*ince the 18th Century, or even before, many scientists have dedicated their professional careers to determining protein requirements and recommendations for humans **(Figure 1)**. The first recorded evidence of protein requirements and recommendations appeared in ~1877 and was credited to Carl von Voit who was a German physiologist and dietitian. Von Voit made the recommendation that a 70kg person whom undertakes a *'moderate'* level of work should consume 118g of protein per day and referred to this value as the '*lowest limit*' of supply to avoid risk of '*damage to health*'(23,24). This figure was devised despite a dietary survey carried out in Munich by von Voit, that suggested a protein intake of 52g per day was sufficient for good health (later, in ~1900, von Voit would recommend a protein requirement of 1.0g per kilogram of body weight per day based on the dietary intake of highly productive factory workers) (23,24). In contrast, at the beginning of the 20th Century, supporters of nutritional reform recommended a daily protein intake of <30g per day. A key representative of nutritional reform was the Danish nutritionist, Mikkel Hindhede, who conducted experiments demonstrating long-term adherence to diets with a daily protein intake of <30g per day(25). Hindhede also suggested that earlier estimates of >100g per day were exaggerated and highlighted the observation that recommendations were based on non-animal foods that were considered '*less protein dense and cheaper than a meat-based diet*'. As such, these recommendations were claimed to have helped avoid famine during World War 1(25).

During the 20th Century, with significant advances in science and communication, a concerted effort was made by international committees to devise universal guidelines for protein intake recommendations. While the originally proposed daily allowance of 1.0g of protein per kilogram of body weight for adults represented a figure of appealing simplicity, this recommendation was not based on scientific evidence. Accordingly, in 1955 the Food and Agriculture Organization (FAO) assembled a committee, led by Professor Emile Terroine, to define the average/minimum requirements and the recommended allowance for dietary protein (see below for definitions of each) (26). The average requirement for protein intake was set at $0.35 g \cdot kg^{-1} \cdot day^{-1}$ for adults. Protein requirements and recommendations were revisited in 1963 by a Joint FAO/World Health Organization (WHO) Expert Committee(26), with an average protein requirement of $0.59 g \cdot kg^{-1} \cdot day^{-1}$ agreed that factored in nitrogen losses and the additional requirements for growth.

The FAO/WHO Expert Committee reconvened on multiple occasions in the years that followed to continue to refine protein recommendations, which included, for a brief period, sex-specific guidance (0.44 and $0.40 g \cdot kg^{-1} \cdot day^{-1}$ for men and women, respectively). In 1981, a joint FAO/WHO/UNU Expert Committee calculated the mean protein requirement based on short-term and longer-term nitrogen balance studies (this technique is discussed below) and concluded no clear evidence of sex differences in nitrogen losses and thus protein requirements or recommendations(26). The average requirement for highly digestible, good-quality protein (e.g., meat, milk, fish, egg) was set at $0.60 g \cdot kg^{-1} \cdot day^{-1}$ for both sexes. To translate this estimate of the average protein requirement to a level sufficient to cover individual variation within a population group, an estimated value of 2 standard deviations above the average physiological requirement would be expected to meet the needs of the majority of the population. Hence, the lower end of the safe intake of good quality, highly digestible protein was therefore set at $0.75 g \cdot kg^{-1} \cdot day^{-1}$. In 2007, and informed by a meta-analysis of nitrogen balance studies, a Joint FAO/WHO/UNU expert consultation, recommended $0.83 g \cdot kg^{-1} \cdot day^{-1}$ of protein to meet the requirements of most (97.5%) healthy adults(27,28) (also see Rand *et al.*, 2003(29)). To this end, these data provide the fundamental evidence base which informs protein requirements and recommendations by relevant authoritative bodies.

Amino acid requirements: taking dietary protein requirements and recommendations one step further?

The concept of devising AA, in addition to or instead of protein, requirements and providing specific recommendation for each EAA is appealing given that not all dietary protein sources contain an identical AA profile. However, this concept is challenging to implement in practice. Hence, recommendations for intake of specific AA have been limited, as discussed elsewhere(30,31). The concept of AA requirements is ostensibly based on knowledge that the EAA content of a protein source, rather than the gross protein *per se*, dictates the metabolic availability and ‘quality’ of a protein source, with implications for muscle anabolic potential, and must be ingested in the diet. A seminal rodent study in the early 20th century revealed low survival rates in rats fed a diet exclusively containing zein (derived from maize/corn which constitutes an ‘incomplete’ low-quality protein, deficient in lysine and tryptophan) compared with rats fed casein from cow's milk, a high-quality protein with a full complement of EAA. Through a series of investigations(32–35), this led biochemist and nutritionist, Professor William Cumming Rose, to the discovery of the EAA threonine(32–35). Through manipulation of rodent diets, Rose demonstrated that 10 amino acids are essential for rats and have to be consumed via the diet as they cannot be synthesised in sufficient amounts without dietary intervention. Follow up work demonstrated that 8 amino acids are essential for adult humans (isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine). Longer-term studies established histidine as essential for adult humans, bringing the total to nine (and eleven non-essential amino acids)(36). In brief, Rose's human experiments involved the provision of rudimentary diets to healthy male graduate students, consisting of corn starch, sucrose, butterfat without protein, corn oil, inorganic salts, the known vitamins, a large brown "candy" made of liver extract flavoured with peppermint oil (to supply any unknown vitamins), and mixtures of highly purified individual AA. In addition to nitrogen balance data to support his conclusions, Rose also noted a higher prevalence of symptoms of nervousness, exhaustion, and dizziness when participants were deprived of an EAA(33). Although Rose's work received some criticism including concerns over the validity of prescribed diets, his findings remain fundamental to our current understanding of human AA requirements and human protein metabolism. Accordingly, subsequent research revealed that only EAA are required to increase MPS(37). Notwithstanding, whilst all EAA must be obtained through diet, even when not acquired acutely (i.e., during a single meal), a true AA deficiency is difficult to achieve longer-term via a habitual diet which likely contains a variety of different proteins and wholefood sources to an extent that complete deficiency is avoided(38). The key factor(s) that discerns an EAA from a NEAA in humans remains to be fully established, but is likely attributed to a combination of evolutionary mechanisms and as a means to regulate energetically expensive cellular processes (e.g., MPS)(39,40). Moreover, there is no evolutionary advantage for the endogenous generation of EAA, as they are sufficiently available through a "standard diet", and circumvent the need to use long, complicated, and energy consuming pathways that would be required to synthesize sufficient quantities of all EAA.

Nitrogen balance: determining protein requirements in humans

The requirements for EAA and thus dietary protein have been determined by multiple methods to inform protein requirements and recommendations. Historically, descriptive or gross measures including growth and nitrogen balance have been used. To this end, the estimated average requirement (EAR) and recommended daily allowance (RDA) (discussed below) have been determined by the single endpoint of the amount of protein intake required to maintain nitrogen equilibrium (namely food nitrogen intake minus nitrogen excreted [urine, faeces, sweat skin and hair]), otherwise referred to as 'nitrogen balance'(41). However, concerns have been raised regarding the use of this technique for determining protein requirements, not least that recommendations are based on good quality protein(29) and that readouts of nitrogen balance has limited utility beyond nitrogen balance itself which lacks sufficient physiological relevance to outcomes related to lean body mass(42). In brief, nitrogen balance requires a minimum of 3 days per level of test intake (i.e., amount of dietary intake of protein) and 7–10 days of adaptation are needed to each intake of protein(43). In addition, complete collection and quantification of all sources of nitrogen excretion, mostly in urine and faeces, are required but this is practically challenging. Moreover, the nature of the nitrogen balance calculation is often associated with significant variability given that nitrogen intake and excretion are independently associated with significant error, thereby lacking sufficient sensitivity(42).

The validity of the nitrogen balance technique has also been criticized given that a zero nitrogen balance on a lower protein intake may reflect biological accommodation (i.e., individuals can adapt to insufficient/suboptimal protein intakes by reducing nitrogen excretion)(42,44–46). In addition, studies have demonstrated an apparent disconnect between positive nitrogen balance and projected improvements in lean body mass(41,42). Clearly, there are several limitations and additional considerations associated with the nitrogen balance technique that question the validity of current estimates of protein recommendations(41,42,47). Indeed, even as early as 2002 the 'dietary reference intakes' report from The Food and Nutrition Board of the Institute of Medicine (The National Academies) stated that "*due to the shortcomings of the nitrogen balance method, it is recommended that the use of nitrogen balance should no longer be regarded as the 'gold standard' for the assessment of the adequacy of protein intake and that alternative means should be sought*" (Institute of Medicine of the National Academies)(41). In contrast, recent data suggest that nitrogen balance may be useful in detecting EAA deficiencies in low intake states given that consumption of the protein RDA (~$0.80 g \cdot kg^{-1} \cdot d^{-1}$) following a strict, low-quality protein, vegan diet for ≥1-year has been shown to be inadequate to achieve nitrogen balance(48). Furthermore, the reanalysis of previously published nitrogen balance data, when using a different analytical approach (via 2-phased linear crossover analysis), revealed a higher population estimate of $1.0 g \cdot kg^{-1} \cdot d^{-1}$, which approaches the protein requirement determined using more contemporary methods(49).

As a potential alternative to nitrogen balance for determining protein requirements, the Nitrogen-15 (^{15}N, a rare stable isotope of nitrogen) End-Product method has also been proposed(50,51), a technique that has been employed for >50 years to measure the turnover of the entire nitrogen pool of the body(51). In brief, the ^{15}N End-Product method involves the oral ingestion of a labelled nitrogen (e.g., ^{15}N-glycine, ^{15}N-alanine) to determine nitrogen flux, or nitrogen turnover at the whole-body level. This method is based on the assumption that metabolically active nitrogen is freely exchanged between nitrogen-containing tissues and the metabolic nitrogen pool (e.g., amino acids)(52). Nitrogen appearance in the metabolic pool occurs exogenously via the diet and endogenously via protein breakdown with nitrogen disappearance occurring through protein synthesis and nitrogen excretion as end-products, primarily urea or ammonia in the urine(53). Measurements of whole-body protein breakdown, in addition to synthesis, can also be calculated by measuring protein intake. However, similar to nitrogen balance, this technique is associated with measurement error and technical challenges. The calculation of nitrogen flux, protein synthesis, protein breakdown and net protein balance using this technique are described elsewhere(51,54,55).

Contemporary approaches for determining whole-body protein requirements

A more contemporary and arguably comprehensive method to determine protein requirements is called the indicator amino acid oxidation (IAAO) technique(42,56–58). The most common application of IAAO is to provide an oral AA mixture to human subjects. Using IAAO, an EAA is ‘labelled’ with a stable isotope (usually ^{13}C) and the appearance of this label in the breath (carbon dioxide, $^{13}CO_2$) is used to quantify AA oxidation as an indicator of protein or a single EAA requirement. IAAO was developed based on the principle that all EAA are required in sufficient quantities for protein synthesis. In theory, if a single AA is limiting or provided in excess, AA oxidation will be observed. Stable isotopes are naturally occurring atoms (e.g., carbon, oxygen, nitrogen, sulphur) containing extra neutrons, whose metabolic fate replicates their more common isotope, permitting a distinction between common and rare isotopes that are detectable (or ‘traceable’) in biology. Similar to nitrogen balance, the IAAO technique provides subjects with graded protein (or AA) intakes across multiple trials during which the *indicator* AA is provided at a continuous, excess, amount, and adaptation of only 3-4 hours is required(59). When the intake of protein/AA is low, the availability of one or more EAA will be limiting for protein synthesis, and thus will be oxidised. As protein intake levels increase, the excess and thereby the oxidation of the indicator AA decreases, reflecting an increased incorporation of AA into protein. The AA intake level at which AA oxidation becomes minimal is termed the ‘breakpoint’ and represents the intake level that maximises whole-body protein synthesis rates. The same concepts apply for the assessment of EAA requirements, except that graded amounts of the EAA are provided while all other AA are provided in excess(3). Fundamentally, this technique is based on the principle that beyond lean tissue itself, there is no inactive compartment to serve as a reservoir for AA and therefore AA must be partitioned between incorporation into protein or oxidation.

Evidence from the application of IAAO suggests that current recommendations for dietary protein may underestimate minimum protein requirements for whole-body balance by as much as 50%, including in older people(43,60–63). Indeed, a recent review of the literature suggests that protein requirement estimates using the IAAO method range from ~5%–260% greater than the RDA across a range of populations(58). A key criticism of IAAO is that participants are only adapted to the test intake on the study day, however, adaptation to longer periods does not seem to impact estimates of dietary requirements(56,64). In addition, it also is feasible that oxidation (and thus IAAO) reflects fluctuations in protein synthesis only rather than protein breakdown(56) that serves as a key component in accurately determining net protein balance, albeit less critical in healthy adult populations(65,66). Clearly, current protein recommendations warrant consideration in the context of best available tools to provide valid estimates of required intakes, and this may be achieved with the employment of multiple assessments including IAAO. Understanding the specific EAA requirements across the health- and lifespan continuum and the provision of easy-to-access resources relating to dietary protein sources is of particular interest, particularly in the context of healthy ageing. Moreover, other emerging methods to measure protein kinetics may be suitable for estimating protein requirements including the use of deuterium oxide (heavy water) and D3-Creatine, but require investigation to confirm their utility in accurately determining protein requirements and recommendations in a range of populations(51,67).

Current UK recommendations for dietary protein intake: a misunderstood concept?

Formalised dietary protein recommendations have been devised for >100 years(68). Nonetheless, optimal and/or recommended protein intakes across the health- and lifespan remain unclear(4). The current UK Recommended Dietary Allowance (RDA) for protein intake is based on a normal distribution of population requirements and an estimated average requirement (or 'EAR', satisfying the requirements of ~50% of the population) of ~0.55-0.60$g \cdot kg^{-1} \cdot d^{-1}$, and is set at 0.75$g \cdot kg^{-1} \cdot d^{-1}$ for healthy adults (~50-55g per day for a 70-75kg individual). The general purpose of the RDA, which is set at the EAR plus two standard deviations, is to meet basic nutritional requirements and avoid deficiencies in 97-98% of the population. Nevertheless, the protein RDA can easily be misrepresented and misinterpreted(41). Indeed, the protein RDA is not a 'recommendation' nor an 'allowance', but rather an 'adequate intake amount' to avoid a negative nitrogen balance in the majority of the population(41). This notion creates a further problem in that, unlike other macronutrients, the RDA for protein is not based on a health outcome (e.g., association with disease, function, lean tissue mass). Based on its definition, the protein RDA is therefore not intended, nor does it provide, an estimation of 'optimal' intakes, or exclude the possibility that less than the RDA represents a sufficient or optimal intake for a given individual.

In addition to the RDA, the Acceptable Macronutrient Distribution Range (AMDR) for protein is set at 10-35% of total caloric intake and was developed to express dietary recommendations in the context of a complete diet. However, in isolation the AMDR is not considered helpful for dietary guidance. Indeed, the lowest level of protein intake reflected in the AMDR is higher than the RDA (when reference body weights of 57kg and 70kg are assumed for women and men, respectively)(41,69). In addition, if an individual were to meet the RDA for all macronutrients, only ~40% (depending on age, sex, activity level, and other factors) of the total energy requirement would be met, highlighting a wider issue with macronutrient recommendations(41). Moreover, protein recommendations are not typically further delineated on the basis of other characteristics (e.g., age, sex, activity level, health status [exceptions discussed below]), despite data suggesting specific health benefits at levels of protein intake that significantly exceed the RDA(70,71). Based on its purpose and definition, the protein RDA *may* more appropriately be termed the "*recommended minimum intake*", alongside recommendations to increase daily intake, as previously proposed(41). However, we would apply some caution to this recommendation as the RDA, or below, may represent a level of intake that is optimal for a proportion of the population. Indeed, a population-wide recommendation to increase the protein RDA, or at least a suggestion that the RDA is the absolute minimum, may not be sensible for individuals with existing kidney damage, whether this condition is formally diagnosed or is unknown. The discussion of personalised recommended *vs*. optimal *vs*. maximal protein intake(s) is an important consideration(4,70,72,73). Undoubtedly, numerous factors warrant consideration when devising protein recommendations across the health- and lifespan continuum and, where possible, a tailored approach to protein nutrition should be considered as part of a well-balanced diet to supply the increasing demand of specific nutrients associated with ageing to avoid malnutrition(74).

Refining per meal protein recommendations for skeletal muscle anabolism in older age

The primary metabolic regulator of skeletal muscle mass is the stimulation of MPS and has been shown to correlate with longer-term changes to skeletal muscle outcomes(75). The use of stable isotope methodology to measure the acute response of MPS to a single protein bolus has provided the scientific foundation to refine protein recommendations on a per meal basis. In healthy young adults, close to a consensus has been reached that a per meal dose of ~20–30g (~0.25–0.30$g \cdot kg^{-1}$) of high-quality protein (equating to ~3g leucine; ~10g EAA; ~5g BCAA) is sufficient for the maximal (but transient; ~2–5h) stimulation of MPS. However, the AA composition, specifically the EAA profile and leucine content (the intracellular appearance of which seems particularly important for the stimulation of MPS(76)) of the protein source will ultimately influence the required protein dose for the maximal acute stimulation of MPS(77). Further, whilst young individuals demonstrate a robust response of MPS to these anabolic stimuli, a blunted response has been observed in older adults, termed 'anabolic resistance', which likely underpins muscle loss observed with ageing(71,78). For example, Moore and colleagues (2015)(71), performed biphasic linear regression and breakpoint analysis using data sets derived from multiple laboratories that measured the acute response of MPS after the ingestion of varying amounts (0-40 grams) of high-quality dietary protein (as a single bolus) in healthy older (mean of 71 years) and younger (mean of 22 years) men when normalized to body mass(71). Whilst no difference in basal postabsorptive MPS rates were observed between age groups, biphasic linear regression and breakpoint analysis revealed the slope of first line segment was lower in older men and that MPS reached a plateau after ingestion of 0.40 ± 0.19$g \cdot kg^{-1} \cdot meal^{-1}$ (95% CIs: 0.21-0.59$g \cdot kg^{-1} \cdot meal^{-1}$) and 0.24 ± 0.06$g \cdot kg^{-1} \cdot meal^{-1}$ (95% CIs: 0.18-0.30$g \cdot kg^{-1} \cdot meal^{-1}$) in older and younger men, respectively. These data suggest that older adults may require almost 2 × the per meal dose of protein to achieve a comparable MPS response to their younger counterparts(71). Moreover, the large overlapping confidence intervals (0.21-0.59g·kg^{-1}·meal^{-1} and 0.18-0.30g·kg^{-1}·meal^{-1} for older and young, respectively) highlight the inherent biological variability in MPS response to ingested protein, particularly with advancing age, suggesting personalised protein recommendations regardless of age, are warranted when devising future protein recommendations. However, it is worthy of note that whilst protein intake is an independent, albeit small, predictor of better retention of muscle mass in older age, exercise represents the main stimulus for muscle adaptative remodeling, particularly resistance exercise(17,79–83). Therefore, even in scenarios where alternative protein recommendations are reached, this could elicit only a small effect on muscle anabolism and remodeling in the absence of resistance exercise(17,79–83). In addition, it is important to caveat that these findings presented by Moore and colleagues(71), and others, are predominantly isolated to skeletal muscle and, even more so, the myofibrillar (i.e., contractile) proteins within skeletal muscle (largely from quadriceps muscle). Hence, these observations typically reflect the acute, fasted response to high-quality liquid forms of isolated protein.

Optimising protein nutrition for muscle health can be more complex than simply recommending a daily total protein intake (e.g., source, type, quality, timing, pattern, nutrient co-ingestion). As a logical extension to per meal protein recommendations, the notion that daily protein intakes should be spread evenly between meals/servings (~3–4 hours) is intuitive, particularly in older adults that typically consume the majority of their daytime protein intake within a single meal(84). Indeed, a common proposal based on the ‘refractory period’ (or ‘muscle full effect’) of MPS(39) and that there is no inactive compartment to serve as a reservoir for protein, is that an even daily protein intake distribution across feeding events is superior to an uneven skewed distribution. However, conflicting findings have been reported from studies in older adults that have measured the response of MPS and lean mass outcomes to the manipulation of protein meal pattern(85–89), with some indications that meal 1 (i.e., breakfast) is when muscle seems to be the most receptive to protein provision, as during sleep recycled AA are directed toward more critical organs and away from skeletal muscle(85–89). Accumulating evidence, though, also suggests that bedtime protein feeding may increase overnight MPS rates and enhance skeletal muscle remodeling(90). However, given that most of our understanding of MPS responses to protein provision is based on isolated protein sources, particularly in the acute postprandial phase, caution should be applied when translating to longer-term, habitual practices which consist predominantly of wholefoods of varying ‘quality’. Nevertheless, based on current understanding, it is generally accepted that recommended protein intakes for, especially active, older adults should exceed the current RDA and be raised to 1.0-1.2g·kg^{-1}·day^{-1} based on 3 × ~0.4g·kg^{-1}·meal^{-1}(91). Further, wholefoods are typically nutrient-dense and better represent habitual dietary patterns than isolated protein sources. Unlike isolated sources, protein-rich wholefoods contain other non-protein derived nutrients that theoretically may affect the stimulation of MPS, although this area of research is in its infancy. Nevertheless, the preponderance of data suggests that protein-rich wholefoods do not inhibit the MPS response(92) and, combined with the pragmatism of having to account for ‘other’ nutritional needs, we would therefore recommend that the majority of an individuals’ protein intake should be derived from wholefood sources, where possible.

For >20 years there has been suggestions that the RDA for protein may not be adequate for older people to maintain skeletal muscle(45). Whilst these guidelines markedly exceed the RDA, there is currently no evidence that high(er) protein diets are harmful to health (e.g., kidney, bone) in otherwise healthy individuals(93–96). Numerous studies in older adults support the notion of longer-term higher (than the RDA) protein intakes on lean mass outcomes (e.g., lean body mass, muscle mass, bone health, metabolic health, body composition, strength, function)(17,97–103). Furthermore, a series of studies have observed no harmful effects on blood lipid profiles, metabolic health, liver or kidney function when prescribing very high ($3.4–4.4 g \cdot kg^{-1} \cdot day^{-1}$) protein diets for periods of up to 6 months, albeit in resistance-trained individuals(104–107). Notwithstanding, we acknowledge that achieving these high(er) protein intake recommendations can be challenging, particularly for older adults. Indeed, one in three older adults fail to consume even the protein RDA(74). This protein undernutrition is exaggerated in frail older adults owing to issues such as reduced appetite, dysphagia, medications and/or psycho-social barriers. Moreover, a low protein intake is associated with frailty(108). The consumption of high-quality protein foods and liquids, protein supplementation and/or fortification of foods increases the peripheral availability of dietary AA and thus represents a potentially effective strategy for compromised older populations that warrants further exploration. Indeed, multiple factors can impact the likelihood of malnutrition and our nutritional (and, specifically, protein) needs and these must inform interventional dietary approaches and dietary protein intake recommendations in older adults(109).

Alternative protein sources for muscle protein synthesis in the 21st Century

To date, formal protein recommendations have almost exclusively focussed on protein dose with relatively limited consideration to protein source or quality. In contrast, perhaps the most significant evolution in protein recommendations relates to the transition from typically higher-quality animal-based to typically lower-quality plant-based protein sources. This trend is driven, at least in part, by increasing concerns surrounding the sustainability of animal-based protein production to meet growing global population demands(110). Protein quality is defined by a number of factors, including the AA content (particularly leucine), AA profile and AA bioavailability combined with protein and/or AA needs, and the digestion kinetics and delivery of AA to biological tissues for protein synthesis(111,112). Historically, animal proteins have been considered to stimulate a greater postprandial MPS response and thus superior for muscle anabolism, largely due to their relative high ‘quality’ (i.e., composition of EAA), high density of protein (i.e., proportion of protein per total weight) and high digestibility. Indeed, early records of protein recommendations refer almost exclusively to animal-based products as “highly digestible and good-quality protein”, while highlighting the need to consume more foods to reach protein requirements if derived from non-animal-based “less protein dense” sources. Consistent with this notion, some previous studies suggested that plant proteins were less potent in stimulating MPS compared with animal proteins at an equivalent dose(111). This notion was assumed to be attributed to the typically lower EAA content, limited content of a specific AA such as leucine, lower digestibility, and/or higher splanchnic extraction of AA of plant proteins(113,114). However, these potential issues can be overcome relatively simply via protein extraction, AA fortification, protein blends that exhibit complementary AA profiles and/or simply increasing protein intake to meet AA requirements(113,114).

A growing body of research has demonstrated that animal-free protein sources can effectively stimulate MPS in a manner that is comparable to animal-based proteins(113,115–118), although this observation is likely to be context dependent. Indeed, at least in young ‘anabolically’ sensitive adults, even when a less favourable increase in plasma bioavailability (i.e., lower postprandial plasma AA) have been observed following the ingestion of non-animal compared with animal protein sources, markers of skeletal muscle anabolism are comparable(119). However, the application of an exclusively plant-based lower-quality protein diet may be concerning if insufficient quantities of protein (and thus EAA) are consumed. This deficiency is exacerbated by the observations of reduced peripheral availability of AA with ageing (via increased splanchnic retention of AA(120)) which likely contributes to age-related muscle loss(120). Indeed, increased splanchnic retention of AA is also associated with plant-based proteins, due to their lower digestibility(118,121,122). It is, though, worthy of note that whilst the impact of insufficient provision of all EAA may be difficult to detect in tightly controlled acute metabolic studies, an accumulation of small AA deficiencies over an extended period of time may be important and result in a greater cumulative MPS deficit, with consequences for skeletal muscle health(123), as muscle breakdown, and thus atrophy, will likely need to increase to provide an endogenous supply of EAA for critical physiological tissues and organs(66,124). Nevertheless, in practice, humans rarely consume foods in isolation and mixed meals within a habitual diet likely contains sufficient amounts of all EAA. Based on current evidence, if protein intake is $\geq 1.6 \mathrm{g} \cdot \mathrm{kg}^{-1} \cdot \mathrm{day}^{-1}$, the long-term impact of protein source (within a mixed whole food diet) on muscle remodeling may be negligible(111). Indeed, for most people, the benefits of protein intake and different protein intake strategies seem to diminish greatly beyond $\sim 1.6 \mathrm{g} \cdot \mathrm{kg}^{-1} \cdot \mathrm{day}^{-1}$(7,111).

Although largely speculative, it should be considered whether there are metabolic and molecular consequences of switching to an exclusively plant-based lower-quality protein diet in older age, having followed an omnivorous diet throughout the majority of an individual’s life. Indeed, individuals habituated to high protein, and thus high EAA, intakes may require a greater relative protein intake to account for an attenuated peripheral dietary AA appearance and/or enhanced AA oxidative capacity(44) given that processes involved in the uptake of AA into muscle may be more efficient under scenarios of an impaired muscle anabolic potential(125). Whilst there is currently limited evidence to support any long-term detriment of a plant-based diet on musculoskeletal outcomes at an advanced stage of life(126,127), it is important to note that humans possess inherent adaptive biology which provides an evolutionary advantage(128,129), and raises the question, is nature smarter than people think? Hence, we cannot exclude the possibility that the same cannot be true for longer-term exposure to types of protein source, under conditions of chronic protein ingestion from lower- or higher-quality sources.

Sustainability of different protein sources: a complex debate

Alternative protein sources cannot be discussed without an acknowledgment of and appreciation for environmental sustainability. Much controversy and misinformation surround the sustainability associated with our food choices. Undoubtedly, rapid growth in global population has contributed to stressors in food systems that have clear consequences for the environment and the continued existence of our planet(130). Indeed, concerns surrounding the sustainability of increased production of animal-based proteins to meet growing consumer demands is driving nutritional research into alternative protein sources (e.g., plant, fungal, algal, insect, laboratory grown 'meat', 'animal-free animal proteins'), which will represent an area of intense research for many years to come(110). A reductionist approach to this issue is to advise a global population switch to excessive plant-based diets(131), however, the sustainability of different protein (and food) sources is a hugely complex debate for multiple reasons. First, dietary protein sources differ by many characteristics (e.g., AA composition, digestion characteristics, protein density, nutritional composition, form) that justifies the need for assessments of environmental impact to include nutritionally relevant functional units(132–134). Indeed, a recent study suggests that, whilst their analysis revealed animals source foods still tended to be associated with higher environmental impacts than plant-based foods, shifting to a nutritionally relevant functional unit in life cycle analyses confirms a lower relative environmental impact of nutrient-dense foods compared with when using conventional units (e.g., per total weight, calories) (135,136). Further, when considering 'ounce equivalents' of protein food sources, which is a recommendation published by The Dietary Guidelines for Americans to help consumers meet protein requirements with a variety of protein food sources, consumption of ounce equivalents of animal-based protein food sources, such as beef, pork, eggs, result in a greater gain in whole-body net protein balance than the ounce equivalents of plant-based protein food sources, such as tofu, kidney beans, peanut butter, mixed nuts, with further inter-individual variations between protein food sources of various types(137). Therefore, protein source, and by extension quality, is an important consideration in the context of fully understanding the environmental consequences of a given food source, which is likely due to distinct differences in nutrient density (i.e., EAA profiles) and bioavailability of EAA for use by the body.

Second, environmental consequences are associated with every stage of the food chain from agricultural production (e.g., farming methods, land use), processing and manufacturing (e.g., packaging, transportation), consumer activities (e.g., storage, cooking) and food waste disposal, and these consequences are not mutually exclusive for protein sources across the spectrum of protein 'quality'(138). In addition, lots of produce goes to waste during processing and transportation due to damage, with some forms of produce more vulnerable to damage than others(139). According to the FAO, ~1/3rd of all edible produced foods are wasted every year across the entire supply chain, accelerating environmental consequences associated with global food production, highlighting the need for immediate urgent alternative action(139). There is growing consensus that food systems need to sustainably provide a diversity of both plant and animal source foods, not least for their protein (and more specifically, EAA) content but other vital nutrients(140,141), to meet global nutritional requirements whilst minimizing environmental consequences(132,140,142,143). Accordingly, several early studies have investigated different means to increase the palatability and quality of protein sources that are disposed of during the food production process. For example, blue whiting and nile-tilapia are underutilised fish species containing high-quality protein and, following hydrolysation, have been investigated for their skeletal muscle anabolic properties using marine by-products that have traditionally been disposed of during production(144,145). In addition, the use of other food sources, including insects, have been proposed as an alternative approach to developing high quality protein with a lower carbon footprint to support skeletal muscle health(146,147). Indeed, the consumption of insects is already common, predominantly in Asia, Africa, and South America, and has gained huge interest in recent years as an alternative dietary protein source that may be produced on a more viable and sustainable scale and, as such, may contribute to global sustainability and food security(146–148). Cell- (or lab-) based meat, sometimes referred to as 'cellular agriculture', is also receiving increasing attention(149,150). However, the current energy cost associated with cellular agriculture is significantly higher than more traditional approaches and the feasibility of this concept to support global demand for food has been questioned(149,150). Undoubtedly, though, some of these approaches do have the potential to maximise sustainability of our food systems to support environmental longevity.

Finally, malnutrition is widespread globally (including protein deficiency(151)) affecting billions of people, with deficiencies higher in lower income countries(136). Diets in higher income countries are typically high in nutrient poor ultra-processed foods, whereas lower income countries diets are dominated by starchy staple (low protein quality and density) foods that lack diversity, each creating their own unique challenges that likely require a nation-specific approach to sustainability and malnutrition(142,152). Further, there is strong evidence to suggest that specific types of foods, including animal foods, are rich in unique nutrients that can otherwise be challenging to consume in sufficient amounts to promote optimal human health in their absence(132,136,141). Indeed, in some of the most prominent 'blue zones' across the globe (i.e., regions where people live significantly longer than the average, often with an extraordinary number of centenarians), whilst diets are often composed predominantly of plant-based foods, they also consist of varying amounts of animal foods that provide vital nutrients that seemingly contribute to extending longevity and vitality. Though beyond the scope of this review, an important consideration in our food choices for sustainability and malnutrition, as well as whole body metabolic health and longevity, is also how the food is prepared and the impacts of modern civilisation on food production, regardless of the source. In addition, approaches such as food fortification may also represent important strategies to combat population nutrient deficiencies(153,154). Undoubtedly, home and/or local produce, land use, food availability, food diversity, less (ultra) processed foods and acknowledging the nutritional value of all foods are all important considerations when addressing food systems in a more holistic manner in line with food demand.

Priority future research directions: where next?

This review has explored some of the most prevalent areas for future research in the field of protein nutrition and put forth some of the key issues and dilemmas that require further research endeavour. Indeed, it is important to recognise the nutritional value of all food types and advocate for foods supported by rigorous, high-quality research that is communicated with policy makers, rather than engaging in polarised public debates. Future research in the field of protein nutrition will likely be dominated by the exploration of novel, alternative, sustainable protein sources that can effectively support skeletal muscle remodeling across the health- and lifespan continuum. Undoubtedly, this new knowledge will encapsulate novel nutrition strategies (e.g., parenteral nutrition, AA fortification) to achieve higher protein intakes in progressively aged and diseased populations. However, as much of our understanding of skeletal muscle anabolic responses to protein are based on isolated liquid-form protein sources, this raises questions over the applicability of current consensuses to habitual practices. Hence, more research is needed into wholefood approaches, including the consumption of ultra-processed foods, that more closely reflect current typical habitual practices. Finally, there is preliminary evidence suggesting that sexual dimorphism to protein provision exists with advancing age. Given the clear gap in female-based research, future work should clarify the sex-specific requirements and recommendations for dietary protein. Undoubtedly, dietary requirements are likely to substantially vary across the globe and indeed across and within clinical populations, and this also must not be ignored when devising future recommendations.

Conclusion

In this review we explored the evolution of human dietary protein intake requirements and recommendations, with a focus on skeletal muscle remodeling to support healthy ageing. Whilst current UK recommendations for dietary protein intake currently sit at ~$0.8 g\cdot kg\cdot^{-1} day^{-1}$, accumulating evidence suggests that, at least in older healthy individuals, we may benefit from increasing these recommendations to >$1.0 g\cdot kg\cdot^{-1} day^{-1}$, which has been verified with the use of more contemporary (e.g., indicator amino acid oxidation) methods to determine protein/amino acid intake requirements. However, recommendations could be refined further to consider other protein intake considerations such as the source, type, quality, timing, pattern and nutrient co-ingestion to provide sufficient essential amino acids for skeletal muscle remodeling. Nevertheless, a growing body of research has demonstrated that animal-free protein sources can effectively stimulate MPS and support skeletal muscle remodeling in a manner that is comparable to animal-based proteins, which have historically been considered superior in their anabolic potency. However, food systems do need to sustainably provide a diversity of both plant and animal source foods, not least for their protein content but other vital nutrients. Undoubtedly, future research in the field of protein nutrition will likely be dominated by the exploration of novel, alternative, sustainable protein sources that can effectively support skeletal muscle remodeling across the health- and lifespan continuum, particularly with wholefood approaches.

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Figure 1 Legend

A brief summary of the key landmarks in the historical evolution of dietary protein and amino acid (AA) requirements and recommendations for humans. Dietary recommendations are provided relative to body weight (i.e., kilogram, kg). EAA, essential amino acids; FAO, Food and Agriculture Organisation; WHO, World Health Organisation; RDA, recommended daily allowance; UNU, United Nations University; EAR, estimated average requirement.

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Conflicts of interest

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Authorship

The talk at the 2023 UK Nutrition Society summer conference, Nutrition at key stages of the lifecycle, was presented by P.M. P.M, as the senior author, and O.W produced the first major draft of the invited review manuscript. P.M, O.W, G.H, D.C, and L.B all contributed to the writing/content of the manuscript. All authors edited and approved the final version of the manuscript and agree to be accountable for all aspects of the work. P.M and O.W produced Figure 1 in Microsoft PowerPoint. “The noblest science is one of making someone healthy”.

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