Alternative dietary protein sources to support healthy and active skeletal muscle aging

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To mitigate the age-related decline in skeletal muscle quantity and quality, and the associated negative health outcomes, it has been proposed that dietary protein recommendations for older adults should be increased alongside an active lifestyle and/or structured exercise training. Concomitantly, there are growing environmental concerns associated with the production of animal-based dietary protein sources. The question therefore arises as to where this dietary protein required for meeting the protein demands of the rapidly aging global population should (or could) be obtained. Various non-animal-derived protein sources possess favorable sustainability credentials, though much less is known (compared with animal-derived proteins) about their ability to influence muscle anabolism. It is also likely that the anabolic potential of various alternative protein sources varies markedly, with the majority of options remaining to be investigated. The purpose of this review was to thoroughly assess the current evidence base for the utility of alternative protein sources (plants, fungi, insects, algae, and lab-grown “meat”) to support muscle anabolism in (active) older adults. The solid existing data portfolio requires considerable expansion to encompass the strategic evaluation of the various types of dietary protein sources. Such data will ultimately be necessary to support desirable alterations and refinements in nutritional guidelines to support healthy and active aging, while concomitantly securing a sustainable food future.
incidence of falls, fractures, and risk of developing chronic metabolic disease.\textsuperscript{4,7,8} Sarcopenia, therefore, underscores the key role skeletal muscle performs in supporting healthy aging by profoundly impacting on both quantity (ie, life-span) and quality (ie, health-span) of life. Consequently, how sarcopenia is managed at complex and interconnected societal levels will dictate the burden it poses to the aging population and global healthcare systems over the coming decades.

**Contemporary management of sarcopenia through physical activity and dietary protein**

A crucial but often overlooked feature of sarcopenia is that, despite being inevitable, the rate of progression is highly modifiable by lifestyle. Research over recent decades has reliably demonstrated that muscle tissue of older adults retains the ability to recondition in response to environmental stimuli such as physical activity and dietary protein intake.\textsuperscript{9–11} Accordingly, adults who retain high(er) physical activity levels throughout their lifespan lose significantly less muscle tissue mass and quality than their (more) sedentary peers.\textsuperscript{5,12,13} Nevertheless, physically active (and even highly trained) older adults still experience some degree of muscle loss that compromises metabolic health and muscle function, and ultimately perpetuates declining health by deterring the continuation of an active lifestyle.\textsuperscript{4,5} Such a vicious cycle can be compounded in those presenting with physical disabilities, frailty, or disease, who have an impeded capacity to carry out physical activity, exacerbated at the greatest extremes by episodic periods of malnourishment and/or muscle disuse during injury or illness.\textsuperscript{14,15}

Typically viewed as an adjunct to physical activity, dietary protein intake has long been considered an additional prerequisite for preserving skeletal muscle mass in older adults.\textsuperscript{16,17} While the minutiae concerning the optimal timing, distribution, amount, etc, of dietary protein intake required to maximize its effectiveness remain under intense scrutiny (eg,\textsuperscript{18–22}), the benefits of combining regular physical activity/muscle contraction with sufficient dietary protein intake on muscle mass, strength, and function in older adults have been demonstrated repeatedly (eg,\textsuperscript{23–29}). General agreement has therefore been adopted by the scientific community that physical activity (or structured exercise training) and sufficient dietary protein intake are the cornerstones upon which recommendations can be built to support healthy muscle aging (eg,\textsuperscript{30–33}). Despite this, however, much less research has considered how the dietary protein source may play into these recommendations. This question has typically been narrowly focused at identifying the most effective source(s) for stimulating muscle anabolism (eg,\textsuperscript{34–35}), but wider and more consequential questions surrounding the efficacy and viability of diverse protein sources, or the potential requirement for supplemental essential amino acids are emerging.\textsuperscript{38} This gap in nutritional understanding appears at a pivotal time societally, while a number of multidisciplinary (and often competing) pressures are forcing us to contemplate where food should be obtained from to best support a sustainable and ethical food future. It should be appreciated that the current knowledge of the impact of dietary protein intake on skeletal muscle of older adults is almost exclusively extracted from studies assessing animal-derived dietary protein sources. This is despite more than half of the total dietary protein consumed worldwide being derived from non-animal protein sources,\textsuperscript{39} coupled with growing interest (particularly in the Western world) in the sustainability and ethical credentials of differing food choices.\textsuperscript{40}

The purpose of this article is to provide a comprehensive and balanced review of what is currently known concerning the impact of consuming dietary protein across a broad range of categorized sources on the skeletal muscle of (active) older adults. This is with the aim of stimulating discussion about how current recommendations, policy, research focus, and clinical practice may be refined to integrate the multifaceted and nuanced issues involved in deciding where dietary protein should (or could) be obtained from, both now and over the coming decades, to support healthier aging.

**Considering the sustainability credentials of dietary protein sources**

From a nutritional perspective, it has been established that meat- and dairy-derived dietary proteins are efficacious sources of dietary protein for maintaining muscle mass in older adults. Indeed, acute metabolic studies\textsuperscript{19,24,34,35,41–43} of muscle protein turnover, and longitudinal or interventional studies\textsuperscript{28,44,45} of muscle mass and function, have used meat and dairy protein consumption in older adults as the crux of the current evidence base, and consequently undergird currently applied (and robust) dietary protein recommendations (eg,\textsuperscript{17,32,33}). However, rising (aging) populations\textsuperscript{46}; increased urbanization, affluence, and economic development\textsuperscript{47,48}; greater awareness of (increased) protein requirements in various populations (eg, athletes, older adults, weight management, clinical situations, etc.)\textsuperscript{17,30,49,50}—among other factors—have converged to explain the rapidly (and presumably exponentially) increasing global consumption of dietary protein.\textsuperscript{51} However, the nutritional evidence base for the efficacy of non-animal-derived proteins to support healthy muscle aging has not kept pace. If not addressed, this
mismatch could result in an imminent threat to the sustainable food future.

The production of animal-derived dietary proteins is typically environmentally costly when quantified in terms of land and water use, and/or the sum of carbon emissions (usually referred to as greenhouse gas [GHG] Emissions), primarily from carbon dioxide (CO₂), nitrous oxide, and methane. Quantifying precise figures here would be complex and politically charged. Estimates are influenced by the diverse methodologies employed, varying criteria for how to quantify embedded carbon within the chain of production, regional differences in food production (due to farming methods [eg, intensive vs extensive], transport methods, crop growing conditions [eg, indoors vs outdoors]), and which variable (ie, land/water use, GHG emission) is afforded primacy. As such, defining environmental sustainability is complex and encompasses various dimensions. Yet, the most globally applied method for quantifying the impact of food and protein production and consumption on environmental sustainability is via the use of GHG emission data, which are consequently the most predominantly available data regarding the environmental impact of dietary protein production. Therefore, in this review, the sustainability credentials of dietary proteins are primarily evaluated through the lens of GHG emissions, while acknowledging that this does not represent “sustainability” in its entirety.

Current estimates lie at around 5%–15% of total global GHG emissions being directly attributable to the production of animal-derived foods. Also, livestock takes up approximately 70% and 30% of global agricultural land and water usage, respectively. In a desire to interrogate to what extent this proportion of GHG emissions could be mitigated by altering food choices, research has attempted to quantify GHG emissions during the production of varying protein sources per kilogram edible food. It is generally considered that animal-derived food production creates considerably more GHG emissions compared with plant-based foods, when considering all the steps and processes involved from farming/cultivation to the availability of the food product in the store (ie, cradle-to-gate). However, most animal-derived food sources, in raw format, especially meat, tend to be more protein dense compared with non-animal alternatives (with legumes an apparent exception), as depicted in Figure 1. Therefore, when assessing the potential role of the various protein-rich sources in developing protein recommendations for muscle mass maintenance, it is appropriate to compare the environmental impact of divergent food sources per gram (or portion) of protein, rather than total food mass or caloric content. To facilitate this comparison, collapsed data from numerous studies has been used to express GHG emissions per 30 g portion of dietary protein (as well as per dose of leucine, branched-chain amino acids, and essential amino acids, with more relevance to the mechanistic regulation of muscle protein turnover being discussed later) (see Figures 2 and 3). Notwithstanding variation depending on specific source and production methods, these data still show that meat is the most environmentally expensive protein source, followed by vegetables and dairy, while fish-derived dietary protein tends to be substantially lower in GHG emissions and at a similar level to plant-based sources. Interestingly, by correcting to protein content, plant-based protein sources generally still encompass a much lower carbon footprint compared with animal sources, especially cereals and legumes, though the differences are clearly less marked compared with the per kilogram edible food comparison. Indeed, the differences even vanish in some comparisons; eg, due to their low protein (and amino acid) density (Figure 1), when corrected per portion of protein, vegetable protein cultivation generates similar GHG emissions compared with dairy. Given the majority of protein supply worldwide is derived from vegetable sources, it is therefore of relevance to evaluate whether increasing this proportion still further would in fact benefit environmental factors but, crucially, also adequately support human nutrition, especially in older adults.

The relative sustainability of plant-based proteins is generally attributed to “cutting out the middle man,” which involves, among others, the inefficiency of growing crops for animal feed, and the consequent increased land use as well as methane production from ruminants’ digestive systems, etc., which together exacerbate the costs across all parameters. For similar reasons—such as: (more) direct culturing (ie, paths of lesser resistance from precursor to product) and controlled, small and enclosed production environments (ie, closed systems), etc.—promising environmental footprints of other (ie, not plant-based) non-animal-derived dietary protein sources such as fungal, algal, and insect proteins are now emerging (Figures 2 and 3). Indeed, the GHG emission intensities of several of these more novel protein sources for human consumption are considerably lower than both animal- and plant-derived dietary protein sources on a per kilogram edible food and per portion of protein (and leucine, branched-chain amino acid or essential amino acid dose) (Figures 2 and 3). However, these sources are less well advanced from a food production, commercial pipeline, and social acceptability perspective, and are therefore minimally consumed compared with animal- or plant-based sources, but they clearly display attractive sustainability and nutritional profiles to warrant further investigation.
While these data (Figures 1–3) shine only a brief light on the complexities of “rank ordering” dietary proteins based on sustainability credentials (a far-reaching multidisciplinary discussion beyond the scope of the present review), they at least underscore the wider societal requirement for us to build a robust evidence base.
as to the nutritional efficacy of non-animal-derived protein sources. Such data are clearly required if divergent protein sources are to be considered and developed to support elevating global protein requirements to support healthier (muscle) aging.

**Figure 3** Mean (± SEM) greenhouse gas emission (kg CO₂e) required to produce a portion of 2.5 g leucine (A), 5 g branched-chain amino acids (B), and 15 g essential amino acids (C) from various categories of whole-food dietary protein sources. White bars represent animal-based dietary protein sources, light gray bars represent plant-based protein sources, the mid-gray bar represents fungal-derived protein sources, and the dark gray bar represents algal-derived protein sources. Values between parentheses indicate the number (n) of included types of dietary protein for which the mean (±SEM) greenhouse gas emission content is calculated. Data are obtained from Table S1. Abbreviations: BCAAs, branched-chain amino acids; CO₂e, carbon dioxide equivalent; EAAs, essential amino acids.

**Skeletal muscle protein metabolism and dietary protein requirements in older adults**

Skeletal muscle mass maintenance in both young and older adults is achieved at the physiological level via a
continuous state of protein turnover, with muscle proteins constantly being synthesized and broken down. Muscle hypertrophy occurs as the result of a net positive muscle protein balance, in which muscle protein synthesis rates exceed breakdown rates over time, and protein is accrued. Skeletal muscle contraction and dietary protein ingestion are the two key anabolic stimuli regulating muscle protein synthesis. Regarding the latter, following dietary protein digestion, amino acids are absorbed in the gastrointestinal tract, resulting in increased plasma amino acid concentrations and, therefore, availability to peripheral tissues, such as skeletal muscle. Once taken up by muscle tissue, dietary protein-derived amino acids can both directly stimulate the signaling mechanisms that upregulate muscle protein synthesis, and can be used as substrate for de novo muscle protein synthesis. Concerning physical activity, it has been established that a single bout of (endurance-, resistance-, or concurrent-type) exercise stimulates mixed (with emphasis of what protein subfraction is most sensitive, contingent on the nature of the exercise) muscle protein synthesis rates for at least 24–48 hours, depending on various factors such as exercise intensity and training status. Importantly, physical activity also sensitizes muscle tissue to the aforementioned anabolic properties of amino acids, which enhances the muscle protein synthetic response to dietary protein. This synergy between physical activity and dietary protein ingestion appears to last for each and every meal following exercise for at least 24 h, and is the metabolic basis for how prolonged dietary protein and exercise interventions can be applied to optimizing muscle tissue maintenance, reconditioning, and/or hypertrophy.

The slow, insidious nature of sarcopenia, as well as its variability in severity and timing of onset, make gross alterations in muscle protein metabolism difficult to detect in older adults. Indeed, most but not all studies do not detect differences in basal (post-absorptive) muscle protein synthesis or breakdown rates between young and older adults. Such findings led to the emergence of the theory that such an imperceptibly small level of daily muscle loss may be better explained by a reduced responsiveness to daily anabolic stimuli. Empirical support followed from multiple studies to show that a blunted muscle protein synthetic response to dietary protein ingestion and physical activity occurs in older compared with younger adults, termed “anabolic resistance.” Such an effect appears most profound at lower (<20 g) compared with higher (>20 g) doses of dietary protein, suggesting a reduced sensitivity of older muscle to dietary protein. The etiology of anabolic resistance is complex and multifactorial, with many physiological effects clearly present upstream, and therefore impacting on the intracellular molecular regulation of muscle protein synthesis downstream. For instance, impairments in protein digestion and/or amino acid absorption, attenuating postprandial amino acid availability, have been reported in older adults. This is likely compounded by increased splanchnic amino acid retention in older adults. Increasing age is also associated with reduced insulin sensitivity, resulting in blunted postprandial microvascular muscle tissue perfusion and, likely, muscle amino acid uptake. While all of these age-related physiological impairments would ultimately result in reduced cellular amino acid supply (both as a signal and substrate) per gram of ingested protein, it is also true that intramyocellular anabolic signaling pathways have been reported as compromised per se in senescent muscle.

Crucially, when viewing the data within the context of healthy and active aging, these different physiological levels of postprandial amino acid handling are all also impaired by physical inactivity. It has been demonstrated that short- and long-term muscle disuse (ie, the removal of muscle contraction) induces rapid muscle atrophy and worsens (or induces) anabolic resistance. Conversely, while (some degree of) anabolic resistance appears inevitable with aging and disuse, physical activity, especially resistance-type exercise, increases the anabolic sensitivity of both young and old muscle, and has the potential to partially overcome anabolic resistance. This likely explains why older adults who maintain an active lifestyle experience lesser declines in muscle mass, and fewer detrimental consequences, than their more sedentary peers, despite equivalent dietary protein consumption. Also, even in the absence of regular physical activity/training, greater amounts of dietary protein consumption appear to overcome, at least partly, anabolic resistance, which clarifies why older adults consuming in surplus of the current Recommended Daily Allowance (RDA) guidelines (0.8 g/kg body weight per day) tend to lose less muscle mass, strength, and functional capacity than aging populations who consume protein at, or below, the RDA. These observations have led to widespread calls within the scientific community to raise dietary protein intake recommendations in older adults up to, at least, 1.2 g/kg body weight per day (eg, ), which is above the current daily protein intake of many older adults in Western societies, and would represent a considerable 50% rise in nationally recognized RDAs. In addition to daily protein intake, factors such as daily protein distribution, or protein intake per meal, may also be optimized to elicit a greater total postprandial muscle protein synthetic response over the course of a day.
Factors affecting the anabolic potential of a dietary protein source

The magnitude of the postprandial muscle protein synthetic response to protein ingestion is regulated across multiple levels. The essential amino acid content and composition of a protein source is an important modulator of the postprandial muscle protein synthetic response. Indeed, the postprandial rise in circulating essential (but not non-essential) amino acids seems to exclusively drive the acute muscle protein synthetic response. Moreover, it has been clearly established that, of the essential amino acids, the branched-chain amino acids (primarily leucine) are particularly important in regulating postprandial muscle protein synthesis rates; however, to prevent substrate limitation, it is generally agreed that all essential amino acids are required to facilitate an optimal muscle protein synthetic response. Leucine not only serves as one of the amino acid precursors for muscle protein, but also as the principle signaling molecule that activates the intracellular muscle protein synthetic machinery. Leucine induces protein translation through direct stimulation of the mammalian target of rapamycin complex 1 (mTORC1), and its downstream effectors (eg, 4E-BP1, pS6K1). As such, leucine content, and the postprandial leucinemia that protein elicits upon ingestion, are of major relevance to the capacity of a protein source to stimulate muscle protein synthesis. Although there are various suggestions that other specific amino acids may also play important signaling roles, it has been shown that simply enriching a single bolus of protein with leucine can further enhance the postprandial muscle protein synthetic response. Collectively, therefore, it is largely assumed that viable protein sources will contain sufficient leucine, and essential amino acids (on a per meal basis) to robustly activate muscle protein synthetic pathways. There is some debate as to what “sufficient” leucine per dose may be, with estimates from 1 g–1.5 g for a measurable increase in muscle protein synthesis, to 2.5 g–3.0 g for an optimal stimulation offered within the literature. “Sufficiency” of leucine is likely modulated by the concordance between the dose ingested and the subsequent efficiency of absorption, such that the amount of leucine within a protein bolus per se is only partially elucidatory. Indeed, this seems to tease apart the subtle difference between the leucine “threshold” and “trigger” hypotheses, with the latter referring to the speed and magnitude of postprandial leucinemia. It should also be noted that there is likely a minimal level required for all (essential and non-essential) amino acids on a per meal protein bolus. Aside from potential (less-well-defined) signaling roles, theoretically the lack of exogenous availability of any amino acid would ultimately become substrate limiting to the continuation of muscle protein synthesis, depending on factors such as postprandial duration and/or endogenous amino acid availability, given that polypeptide chains of complexity require a full complement of amino acids during their synthesis.

Accordingly, viable dietary protein sources are assumed to be well balanced with regard to their amino acid composition, minimizing single amino acid deficiencies as defined by the World Health Organization/Food and Agriculture Organization of the United Nations (WHO/FAO).

As previously alluded to, though amino acid composition is a key initial component influencing the anabolic potential, differing physiological and metabolic aspects of postprandial protein handling will arguably be most consequential. First, dietary protein sources differ in their digestion and amino acid absorption characteristics, leading to divergent (total and kinetic) systemic amino acid availability, which has been suggested to result in different muscle protein synthetic responses to various isolated protein sources. For example, for a given protein dose ingested, total postprandial plasma amino acid availability (ie, the amino acid bioavailability) can vary between sources, which will naturally modulate the resultant muscle protein synthetic response. It has also been shown that rapid aminoacidemia (ie, a quicker and larger “signal”), which occurs when quickly digestible proteins (eg, whey) are ingested, stimulates muscle protein synthesis rates to a greater extent (but possibly for a shorter time) compared with more slowly digested proteins (eg, casein). Though many of these findings are also influenced by the high essential amino acid/leucine contents of whey protein, it has also been shown that...
prehydrolyzing casein\textsuperscript{14} or bolus vs continuous feeding of whey\textsuperscript{146} to accelerate the digestion and absorption kinetics augments the muscle protein synthesis response to the same protein source (ie, identical amino acid composition).

**Dietary protein source and cardiometabolic health during aging**

While it is clear that higher dietary protein intakes are beneficial for maintenance of muscle mass during aging, the broader relationship of how this extends to overall healthy aging requires some nuanced considerations. In general, maintenance of muscle mass with age correlates with decreased morbidity and mortality, and is a predictor of longevity and quality of life.\textsuperscript{7,8,16,13,147} With respect to cardiometabolic health, weight loss tends to be the most notable target, with attention on dietary protein being a key nutritional tool employed during energy-restricted diets. High(er) protein diets (25\%–35\% of total energy intake) have reliably been shown to be effective for supporting losses of body mass and fat mass, while maintaining muscle mass, with resultant favorable changes in blood lipid profiles when compared with standard/low(er) protein diets (10\%–20\% of total energy).\textsuperscript{50,148–150} In addition, dietary protein is also linked with increased satiety when compared with carbohydrate or fat on a calorie-for-calorie basis, permitting a reduced ad libitum energy intake.\textsuperscript{151} The satiating properties of protein have been attributed to, among other factors, postprandial hyperaminoacidaemia,\textsuperscript{152,153} elevated gut hormone concentrations (including concentrations of hormones such as glucagon-like peptide 1, cholecystokinin, and peptide YY\textsuperscript{152,154}), and increased diet-induced thermogenesis.\textsuperscript{155,156} Of relevance, divergent satiating potential has also been attributed to different protein sources. For example, fast-digesting proteins (eg, whey and soy) resulting in transient and high peak concentrations of plasma amino acids and appetite hormones are associated with greater satiety than more slowly digested protein sources (eg, casein).\textsuperscript{152,157} Though satiation of different proteins likely levels out at higher doses,\textsuperscript{158} and the addition of non-protein nutrients (eg, fiber) typically present in protein-rich whole foods also modulates various aspects (eg, chemical, physical, and sensory properties) of food satiety,\textsuperscript{154} evaluating how different protein sources can be applied will be of increasing relevance when considering (sustainable) dietary options for optimal weight loss and metabolically healthy aging.

Much of the relevant data of dietary protein beyond muscle mass per se is out of the scope of the present review, and the reader is referred to the following comprehensive reviews (eg,\textsuperscript{159–162}). However, it would be remiss not to discuss some aspects of the nuanced relationship between muscle mass and muscle quality, and how it relates to dietary protein source. Overweight adults typically have a higher absolute (although not proportional to fat mass) muscle mass, but according to various indices (strength, metabolic function, etc.) the muscle mass is of lower quality, a fact that is of relevance to dietary protein itself and thus protein source selected within the diet.\textsuperscript{160} Emerging epidemiological data have suggested that higher dietary protein intakes are also capable of adversely affecting markers of cardiometabolic health.\textsuperscript{163} This has been extended by studies which report that protein intakes of around 30\% of energy requirements can blunt the improvement observed in insulin sensitivity following 26 weeks of weight loss\textsuperscript{164} and cause a decline in skeletal muscle insulin sensitivity in overweight individuals under eucaloric conditions over 6 weeks.\textsuperscript{165} The beneficial influence of exercise is a noticeable absence, at present, from this emerging literature base, and when accounted for may improve the consistency of the findings.

Of particular interest within the context of the present review, however, is the potential role of the protein source in untangling the relationship between protein intake and markers of cardiometabolic health. For example, it has typically been animal- rather than plant-derived protein sources implicated in negative effects upon insulin sensitivity,\textsuperscript{162} possibly attributed to differences in amino acid composition and content. Indeed, recent studies indicate that the composition of the protein per se may also be of relevance, specifically, the branched-chain amino acid content.\textsuperscript{159,166} Though branched-chain amino acids, as discussed above, play a key mechanistic role in regulating the postprandial muscle protein synthetic response, studies also show their presence in muscle are elevated in human models of physical inactivity,\textsuperscript{167} and in patients with type 2 diabetes.\textsuperscript{168} Further, increased concentrations of circulating branched-chain amino acids (and/or their metabolites) are correlated with insulin resistance and the onset of type 2 diabetes.\textsuperscript{169,170} To date, nutritional intervention data to support this notion are minimal, though vegan participants supplemented with branched-chain amino acids for 12 weeks exhibited reduced insulin sensitivity,\textsuperscript{171} and branched-chain amino acid restriction has been reported to improve insulin sensitivity in rats.\textsuperscript{172} Mechanistic experiments have shown that branched-chain amino acids are capable of acutely impairing glucose tolerance and insulin-stimulated skeletal muscle glucose uptake in various models,\textsuperscript{173,174} in accordance with mechanisms laid out in a “Randle Cycle”–type substrate competition, and/or direct inhibition of insulin signaling by branched-chain amino acid metabolites.\textsuperscript{173,175} These data express the
need for future work to refine dietary protein recommendations for healthy muscle aging in terms of amount, but also to what extent it is consumed against the backdrop of an (in)active lifestyle, as well as the type (and potentially branched-chain amino acid load) of the diet and whether protein source can be leveraged to optimize this.

**Concept of protein “quality”**

The reduced anabolic sensitivity of older muscle to dietary protein ingestion highlights the importance of viewing potential alterations in future RDAs for this age-group through the lens of protein source. To properly evaluate how the origin of a dietary protein source may modulate the recommendations for total intake, the principles of what regulates the muscle anabolic response inevitably falls into making some kind of quantitative assessment over a protein’s “quality” to optimize viable selections for older adults. This is usually achieved by encompassing a protein source’s amino acid composition (relative to human requirements) and its digestibility within composite metrics. The most prominent metrics here are known as the Protein Digestibility Corrected Amino Acid Score (PDCAAS) and, more particularly, the Digestible Indispensable Amino Acid Score (DIAAS), which have been used to rank order protein sources on quality.

PDCAAS determines protein digestibility by expressing the content of the first limiting essential amino acid (the minimal requirement as defined by the FAO/WHO/United Nations University [FAO/WHO/UNU]) of a particular protein against the content of the same amino acid in a reference amino acid composition, based on amino acid requirements for adolescent. The calculated percentage is subsequently corrected for the fecal digestibility of a protein source, as determined in a rat assay. As proteins with PDCAAS > 1.00 were not considered to have any additional value in humans, it was decided to truncate scores at 1.00. Following global implementation of PDCAAS towards the end of the last century, several shortcomings of the PDCAAS metric have been noted. Specifically, fecal digestibility tends to overestimate protein quality, as nitrogen disappearance in the large intestine is predominantly due to microbial degradation rather than protein digestion, and (virtually all) amino acids are absorbed in the small intestine rather than the large intestine. Moreover, PDCAAS does not consider the digestibility of individual essential amino acids, and the use of adolescent amino acid requirements to infer across the lifespan (including individuals at an advanced age) has been questioned. As such, the FAO has recommended the replacement of the PDCAAS with the DIAAS, which is based on individual amino acid digestibility, and estimates protein quality scores using ileal rather than total tract fecal digestibility. Instead of applying rat models, DIAAS uses data involving growing pigs, which is recognized as a more representative model for estimating amino acid digestibility by humans. Lastly, DIAAS does not truncate values at 1.00, allowing further distinction between various “high”-quality protein sources.

While DIAAS estimates amino acid absorption more accurately than PDCAAS, animal models are obviously not fully representative of human protein digestion and amino acid absorption kinetics. Also, both metrics are limited to isolated protein sources, do not consider the influence of the coingestion of other nutrients, or the consumption of dietary protein within a whole food or mixed meal, which modulates post-prandial protein handling and muscle protein metabolism. Additionally, nuanced metabolic characteristics of particular amino acids (eg, leucine) are not accounted for, nor are more holistic systemic effects that modulate the circulating milieu, (eg, insulin secretion, incretin responses, etc.) The limitations of these metrics in determining protein quality as it pertains to muscle anabolism have led to a disconnect when using them to predict its in vivo anabolic potential in human experimentation. For example, despite having similar protein quality scores (ie, PDCAAS ~1.00), soy protein stimulated muscle protein synthesis rates to a lesser extent than an isonitrogenous bolus of whey or milk protein, showing that current protein quality metrics are not necessarily reflective of the post-prandial muscle protein synthetic response. Finally, and crucially, protein demands are constantly influenced contextually. For instance, physical activity, disuse, or aging, and the consequential increased/decreased protein requirements are not captured by protein quality scores. As a result, though the amino acid composition of a dietary protein source and its relative PDCAAS and DIAAS scores may offer a useful foundation when evaluating the viability of protein sources for supporting healthy muscle aging, it is crucial that thorough in vivo human investigation is carried out. Specifically, it is necessary to evaluate the multifactorial physiological and metabolic effects of ingesting differing protein sources on skeletal muscle tissue to determine their true protein quality as it relates to the viability of alternative dietary protein sources. As illustrated in Figure 4, multiple factors are involved in establishing the efficacy of protein sources to support skeletal muscle maintenance and reconditioning, and therefore to assess “true” protein quality. Regarding these factors, an inverse relationship between the amount of (human) data available and its conclusiveness in terms of physiological
endpoints is often observed. For instance, protein composition data (ie, essential amino acid composition and content) is broadly available across all types of protein sources, but provides the least-refined information regarding the in vivo skeletal muscle response. Ultimately, both context (ie, age, and level of exercising) and quality of data are required to assess the utility of multiple types of dietary protein to support healthy aging.

Animal-derived dietary protein sources

Referring to Figure 4; although not complete by any means, datasets pertaining to animal-derived dietary proteins are by far the most complete and robust category. Animal-derived dietary protein sources generally encompass a complete amino acid profile, and therefore are unlikely to elicit any specific amino acid deficiencies that limit muscle protein synthesis rates. For example, dairy, meat, and fish protein sources possess, on average, leucine (8%-9% of total protein), branched-chain amino acid (18%-20%), and essential amino acid (~43%) contents above recommended requirements (set at 5.9%, 12.8%, and 27.7% for leucine, branched-chain amino acid, and essential amino acid contents, respectively) (Figure 5).59,143 Other key amino acids liable for a deficiency (eg, lysine and methionine [see Table S2 in the Supporting Information online]) in other sources are at levels above minimum requirements in most animal-based protein sources. Further, systemic amino acid bioavailability is high following the ingestion of animal proteins. Recent work demonstrated that 65%, 57%, and 45% of intrinsically stable isotopically labelled protein–derived phenylalanine appeared in the circulation following the ingestion of milk, whey, and casein protein, respectively.94 Similar values have been reported for minced beef (61%-64%),41,186 beef steak (49%),41 whole egg (68%),187 and egg whites (66%).187 While equivalently detailed (intrinsically labelled) data are not available for other protein sources, direct comparisons of postprandial amino acid availability following ingestion of animal vs non-animal proteins have clearly supported the contention of animal protein exhibiting high (essential) amino acid bioavailability, suggested as being due to superior protein digestibility as well as lower rates of essential amino acid extraction by splanchnic tissues.41,94,125,186,187 As a result of amino acid composition and bioavailability data, animal-
derived dietary proteins typically score highly on indices of protein quality. As can be seen from Table 1, meat protein typically scores at 1.00 and approximately 1.10 on PDCAAS and DIAAS, with dairy proteins scoring at 1.00 and 1.16, respectively.

It is therefore not surprising that in vivo evidence over the last 20 years has repeatedly and extensively demonstrated that animal-based protein sources robustly stimulate (different subfractions of) muscle protein synthesis rates in the acute postprandial (resting or post-exercise) period in both young and older individuals (eg, 34–36, 42, 79, 187, 202, 203). Indeed, the extensive literature base that exists has enabled researchers and practitioners to select divergent sources to manipulate...
the rate of digestion and absorption kinetics, depending on the application. For instance, “fast” proteins such as whey have been successfully applied to maximize post-exercise muscle protein anabolism, while slower proteins (eg, casein) have been used pre-sleep to provide a more sustained amino acid precursor for optimizing overnight (post-exercise) muscle protein synthesis rates and subsequent recovery.

Table 1  PDCAAS and DIAAS of various protein sources

| Protein source | PDCAAS | DIAAS | References |
|----------------|--------|-------|------------|
| **Animal**     |        |       |            |
| Dairy          |        |       |            |
| Egg            | 1.00   | 1.16  | Ertl et al (2016) |
| Cow milk       | 1.00   | 1.16  | Ertl et al (2016) |
| Fish           |        |       |            |
| Mackerel       | 1.00   | –     | Boye et al (2012) |
| Tuna           | 1.00   | –     | Suárez López et al (2006) |
| Meat           |        |       |            |
| Beef           | 1.00   | 1.12  | Ertl et al (2016) |
| Chicken        | 1.00   | 1.08  | Ertl et al (2012) |
| Lamb/sheep     | 1.00   | 1.17  | Ertl et al (2012), Suárez López et al (2006) |
| Pork           | 1.00   | 1.14  | Ertl et al (2016) |
| **Plant**      |        |       |            |
| Cereal         |        |       |            |
| Barley         | 0.59   | 0.47  | Ertl et al. (2016) |
| Maize          | 0.47   | 0.42  | Ertl et al (2016) |
| Oat            | 0.57   | 0.77  | Cervantes-Pahm et al (2014) |
| Rice           | –      | 0.64  | Cervantes-Pahm et al (2014) |
| Rye            | 0.59   | 0.48  | Ertl et al (2016) |
| Sorghum        | 0.29   | 0.29  | Cervantes-Pahm et al (2014) |
| Wheat          | 0.45   | 0.40  | Ertl et al (2016) |
| Legume         |        |       |            |
| Chick pea      | 0.84   | 0.82  | Nosworthy et al (2020) |
| Common beans   |        |       |            |
| Black bean     | 0.70   | 0.65  | Nosworthy et al (2018) |
| Faba bean      | 0.58   | 0.54  | Nosworthy et al (2018) |
| Navy bean      | 0.61   | 0.56  | Nosworthy et al (2018) |
| Pinto bean     | 0.66   | 0.61  | Nosworthy et al (2018) |
| Kidney bean    | 0.65   | 0.60  | Nosworthy et al (2018) |
| Faba bean      | 0.58   | 0.54  | Nosworthy et al (2018) |
| Lentils        |        |       |            |
| Green          | 0.51   | 0.49  | Nosworthy et al (2018) |
| Red            | 0.55   | 0.54  | Nosworthy et al (2018) |
| Lupin          | –      | 0.68  | Herreman et al (2020) |
| Pea            | 0.78   | 1.00  | Ertl et al (2016), Guillen et al (2021) |
| Soy            | 1.00   | 0.99  | Ertl et al (2016) |
| Seed           |        |       |            |
| Hemp seed      | –      | 0.54  | Herreman et al (2020) |
| Quinoa         | 0.79   | –     | Boye et al (2012) |
| Vegetable      |        |       |            |
| Carrot         | 0.90   | –     | Suárez López et al (2006) |
| Lettuce        | 0.19   | –     | Suárez López et al (2006) |
| Onion          | 0.47   | –     | Suárez López et al (2006) |
| Potato         | –      | 1.00  | Herreman et al (2020) |
| Spinach        | 0.90   | –     | Suárez López et al (2006) |
| Tomato         | 0.47   | –     | Suárez López et al (2006) |
| **Fungal**     |        |       |            |
| Mycoprotein    | 0.99   | –     | Edwards and Cummings (2010) |
| **Insects**    |        |       |            |
| Beetles        | 0.89   | –     | Yang et al (2014) |
| Mealworm       | 0.82   | –     | Jensen et al (2019) |
| Silkworm       | 0.86   | –     | Longvah et al (2011) |

**Abbreviations**: DIAAS, Digestible Indispensable Amino Acid Score; PDCAAS, Protein Digestibility Corrected Amino Acid Score.
relatively advanced the literature base on animal-derived proteins and muscle protein metabolism has become, at least relative to non-animal-derived protein sources.

Acute laboratory studies of hourly muscle protein turnover rates have ultimately translated to longer term skeletal muscle metabolism and remodeling, including within the context of exercise and aging. For example, deuterated water techniques have demonstrated that animal protein intake or supplementation facilitates high rates of daily and free-living muscle protein synthesis, both at rest and in tandem with exercise in young and older adults.\(^{209-212}\) These findings also correspond with chronic studies demonstrating positive associations between animal protein intake contributing to a greater daily total protein intake in surplus of the current RDA, and the maintenance or gain of muscle mass, quality, function, and associated clinical parameters in aging populations.\(^{28,44,45,115,213-216}\) Such work is now plentiful enough that a series of comprehensive systematic reviews and meta-analyses have been able to show the beneficial effects of increasing dietary protein intake in older adults using animal-derived sources upon muscle mass maintenance and adaptation, with or without exercise interventions.\(^{217-222}\)

**Plant-derived dietary protein sources**

The contribution of plant-derived protein sources to global protein intake is often underappreciated; indeed, plant proteins represent the predominant source of protein consumed worldwide.\(^{39}\) Moreover, there is rapidly increasing popularity of various iterations of plant-based diets,\(^{223}\) and an expanding variety of commercially available plant-based protein isolates or plant-protein products (eg, powders, bars, burgers, meat alternative products, etc.) to support these lifestyle choices. Despite this, relatively little research attention has been paid to the muscle anabolic properties of plant-based proteins. A widely held view seems to exist that plant-derived proteins are less anabolic than animal-derived proteins. This is primarily due to generally lower protein densities per gram of product, but may also be the case on a gram-for-gram of protein basis, due to less favorable amino acid profiles and/or poorer amino acid bioavailability. Plant proteins are typically lower in essential amino acids and less balanced, with most sources displaying at least one essential amino acid “deficiency” (Figure 5).\(^{59,143}\)

The relative lack of in vivo human data concerning muscle protein synthetic responses to various plant-derived protein sources makes consideration of how divergent sources differ in their composition crucial. For example, vegetables are known to be low in protein (<2% of total mass), whereas cereals have a similar protein density to that of dairy (~8%–10%), and seeds (dried) and legumes are particularly protein-dense foods (~21%–26%), equivalent to that found in lean meat (~18%) or fish (~19%) (Figure 1, see Table S1 in the Supporting Information online).\(^{59}\) However, plant-protein-containing food typically contributes to a lesser extent to total energy intake.\(^{59}\) Therefore, obtaining 25%–35% of total energy intake (ie, considered as high protein with respect to total energy intake)\(^{224}\) from (whole-food) plant protein is more challenging than ingesting an equivalent amount of energy from animal-based protein sources, and could lead to excess energy intake, especially in pursuit of optimizing dietary protein intake beyond the RDA in older adults. Protein contents of various commercially available plant protein concentrates or isolates are approximately 51%–81%, a range that is also reported for animal-derived protein concentrates and isolates.\(^{225}\) Even with comparable protein isolates, leucine (5%–9% vs 8%–9% of total protein), branched-chain amino acid (13%–18% vs 18%–20%), and essential amino acid (32%–38% vs ~43%) contents of plant-based protein sources are generally lower when compared with animal-based proteins (Figure 5).\(^{59}\) Several plant-based protein sources are also low/deficient in specific essential amino acids; eg, lysine (eg, cereals, seeds), methionine (legumes, vegetables), or leucine (vegetables), with contents below the recommended WHO/FAO/UNU requirements of 4.5%, 1.6%, and 5.9% of total protein, respectively (Figure 5, see Table S2 in the Supporting Information online).\(^{59,143}\) However, a few exceptions worth mentioning are spinach, pea, maize, quinoa, and lentils because of their balanced, nondeficient essential amino acid profile and relatively high leucine content, especially in comparison with other plant-based protein sources (Figure 5).\(^{143}\) Still, due to low(er) protein/essential amino acid contents in raw forms (particularly per calorie ingested), coingestion of / fortification with specific amino acids (eg, leucine, methionine, lysine)\(^{226,227}\) and/or essential amino acid supplementation,\(^{38}\) or further food processing are viable strategies for optimizing protein intake (without excess energy consumption) from primarily plant-based diets.

Human experiments have reliably demonstrated that plant-derived protein sources display inferior protein digestibility/amino acid bioavailability in comparison with animal-based comparators,\(^{35,37,228,229}\) with pea protein as a notable exception.\(^{197}\) In combination with amino acid content, this results in lower scores on the protein quality indices for plant-derived proteins (ie, PDCAAs and DIAAS of 0.29–1.00; see Table 1). Inferior postprandial plant protein amino acid availability has been attributed to greater splanchnic extraction of plant- than animal-derived amino acids, and higher
amino acid oxidation (ie, ureagenesis) and deamination rates. Greater ureagenesis may be caused by the lack of specific essential amino acids, resulting in an imbalance in the essential amino acid mixture required for (gut) protein synthesis. As a consequence, a greater proportion of free amino acids will be directed to the liver, which will serve as a stimulus for ureagenesis. Also, the naturally occurring presence of antinutritional compounds found in plant proteins (eg, trypsin inhibitors, phytates, and tannins) may further impede protein digestibility, absorbability, and subsequently systemic amino acid availability. Removal of these components by various processing methods (such as roasting, extrusion, soaking, and blanching) has been shown to enhance protein digestibility and absorbability (reviewed in). Contrarily, while removal of such factors appears to positively affect protein digestion and absorption kinetics, it is unclear whether such factors or compounds play a role in the postprandial anabolic response. Moreover, potentiating anabolic effects of various nonprotein factors (including vitamins, minerals, fiber, and other bioactive compounds) have been postulated. For example, fermentation of dietary fibers by gut microbiota results in the production of short-chain fatty acids (primarily acetate, propionate, and butyrate), which have been linked with the postprandial anabolic response. As protein is mostly consumed within a whole food, the presence of such factors should therefore not be neglected, and it raises the importance of thinking of protein concentrates/isolates as distinct from protein within a food, meal or diet.

The translation of findings about the protein quality of plant- vs animal-derived protein sources to in vivo muscle protein synthesis date is broadly in line with what one would expect. What is less often recognized, however, is that this area of research is still in its infancy. To date, comparisons have shown that ingestion of isolated milk proteins (whey, casein, and/or complete milk protein) typically, but not always, elicits a greater acute (ie, 0–4 h) muscle protein synthetic response compared with the ingestion of an isonitrogenous bolus of plant-based protein in both young and older muscle. In the few studies available, consuming a greater amount of plant- vs animal-derived protein seems to “rescue” this response, but not always. These protein-matched comparisons are limited in their diversity, only comparing milk proteins with soy, wheat, or potato proteins—plant-based sources that are considerably lower in essential amino acid content and exhibit lower postprandial amino acid bioavailability compared with milk proteins. As a result, the data thus far are perhaps unsurprising, and leave open important questions, and the urgent need to expand the dataset. For example, it has also been suggested that an inferior anabolic capacity of plant proteins may be due to the presence of rate-limiting amino acids, which could be compensated for by consuming more of the protein. Such a proposition has implications for total protein intake recommendations and, therefore, the sustainability credentials of a given diet. An attractive alternative to simply consuming more plant-based protein is the idea of blending sources to optimize amino acid profiles, an idea supported by data demonstrating that soy–dairy, milk–maize, and milk–wheat blends stimulate muscle protein synthesis to a similar extent to protein-matched dairy-derived protein comparators.

Several key factors remain to be elucidated regarding the postprandial regulation of muscle protein synthesis upon plant-based protein ingestion. For example, it is not clear whether the proposed amino acid deficiencies in many sources exist in vivo, and if so, whether they may explain the lower anabolic potential of plant-derived proteins. If they do, it is not clear whether this is a direct result of differing initial amino acid composition, or an indirect result (due to lower bioavailability of the “same” protein as a result of inferior protein digestibility/absorbability/greater splanchnic extraction). Further, whether such an in vivo amino acid deficiency would represent limitation(s) to the anabolic signaling capacity (eg, due to differing leucine or branched-chain amino acid contents within isonitrogenous doses) or rather substrate limitation for (continuation of) muscle protein synthesis (eg, due to differing essential amino acid contents, such as lysine or methionine, across isonitrogenous sources) has also not been clarified. These are consequential questions that require definitive data, in models of aging and exercise, when determining how to recommend plant-derived proteins to support healthy aging in the face of age-related anabolic resistance.

Studies attempting to translate acute muscle protein synthetic data following plant-based protein ingestion to long(er) term muscle protein turnover measurements are currently restricted to a single study looking at potato protein supplementation over a 14-day free-living period. In this study, the authors showed that potato protein isolate can be used as an effective supplemental protein source to increase daily protein consumption and enhance daily muscle protein synthesis rates at rest and during resistance training (compared with no protein supplementation). However, it remains unclear how this would fare compared with other (animal or plant) proteins. Indeed, the few long-term studies that have investigated skeletal muscle adaptive responses to plant-based proteins have reported mixed findings regarding their anabolic capacities. For example, some, but not all,
chronic resistance exercise training studies in both young and older adults show that plant-protein supplementation (with soy, rice, or pea) or adopting a plant-based diet can facilitate similar adaptations in muscle mass and strength as seen with animal protein sources, although protein intake across these studies was >1.2 g/kg body weight/day, which is considerably more than the current RDA. The apparent disconnect between acute and chronic studies likely relates to the numerous additional variables introduced in longer-term studies, including uncertainties about the anabolic effects of repeated meals, the influence of mixed meals/protein sources, and the minimal number of plant-based sources investigated. As such, it may seem that when ample protein is being consumed, protein quantity rather than quality is key in facilitating skeletal muscle remodeling. However, consuming large quantities of protein is not always practical or feasible in aging populations (due to decreased appetite, underlying conditions, etc.), and it remains unclear whether chronic plant-based protein consumption at lower intakes (ie, <1.2 g/kg body weight/day) would comparably support skeletal muscle remodeling in active older adults.

**Fungal-derived dietary protein sources**

Although dietary protein sources have typically been viewed as a dichotomy between animal- and plant-based origins, other potential categories are emerging. For example, the ability to mass-produce protein-rich biomass from microscale quantities of fungus has been industrially and commercially exploited (with dedicated companies proliferating in recent years) to provide a third category of dietary proteins: mycoprotein. Mycoprotein is a whole food produced by the continuous cultivation of the filamentous fungus *Fusarium venenatum*. Based on the controlled environment within which this fungus is produced, the GHG emissions required to produce the protein within mycoprotein are considerably lower than those for a protein-, or amino acid–matched portion of meat- or dairy-derived protein (Figures 2 and 3), and the production process is more efficient in terms of protein yield per unit of water and land usage than the majority of animal- and plant-derived protein sources, including beef, milk, legumes, and cereal proteins.

Mycoprotein is commercially available in various wet-weight formats (eg, patties, burgers, or sausages). Mycoprotein is currently not available as an isolated protein source, and exists as a relatively low-energy, high-fiber food ingredient, with its fat content predominantly consisting of large unsaturated fatty acids, including omega-3 and omega-6 fatty acids, albeit in relatively small absolute quantities due to the low fat content. Despite representing a whole food, dried mycoprotein is protein (45% of total mass) and essential amino acid (46% of total protein) rich (Figures 1 and 4), and scores a PDCAAS of 0.99 (DIAAS unknown) (Table 1). In addition, mycoprotein can be described as a “balanced” protein source, since it meets FAO/WHO/UNO recommendations for all individual essential amino acids; its branched-chain amino acid content (19.8% of total protein) is comparable with that of dairy protein (20.1%), and higher than that of meat (18.3%), fish (18.5%), and plant-derived protein foods (13.3%–18.0%) (Figure 5, see Table S2 in the Supporting Information online). It has been demonstrated that mycoprotein is digested and/or absorbed more slowly than an isonitrogenous bolus of milk protein, but the total postprandial amino acid bioavailability was found to be comparable. The latter translated to bolus mycoprotein ingestion robustly stimulating resting and post-resistance exercise muscle protein synthesis rates in young men. In fact, mycoprotein ingestion stimulated muscle protein synthesis rates to a greater extent than a leucine-matched bolus of isolated milk protein, which may be attributed to a greater amount of total protein ingested, the specific amino acid composition, and/or, alternatively, its existence within a whole food matrix. Growing evidence is emerging to suggest a potentiating anabolic effect of consuming protein as part of a whole food, presumably due to the presence of other macro- and micro-nutrients and/or other bioactive compounds, and their “nutrient–nutrient interactions” within the matrix. For instance, as highlighted above, short-chain fatty acids produced by gut fermentation following dietary fiber ingestion may modulate (positively or negatively) postprandial muscle anabolism. Interestingly, mycoprotein possesses a different fiber profile than plant proteins, resulting in opposing digestive behaviors, which could potentially contribute to divergent anabolic responses between mycoprotein and plant proteins. This further highlights the need to view the role of protein in maintaining muscle mass in older adults, within the context of consuming whole foods incorporated in the diet. When using mycoprotein as a primary protein source (57% of total daily protein intake) to provide a high-protein (1.8 g/kg body weight/d) vegan diet in exercising older adults, comparable daily muscle protein synthesis rates have been reported when compared with an isonitrogenous omnivorous diet. Collectively, these data demonstrate mycoprotein as a viable alternative protein source for supporting...
(optimal) muscle protein synthesis rates in older adults in accordance with varying lines of reasoning (Figure 4), ie, protein density/essential amino acid composition, digestibility and bioavailability, ability to stimulate resting and post-exercise muscle protein synthesis rates, and its nature as a protein-rich whole food that can feasibly be built into the diet of older adults under free-living conditions to support muscle mass maintenance and/or reconditioning. More work is required to translate this to effecting long-term changes in muscle mass and function, across differing protein intakes and lifestyle interventions, with more direct comparisons with other protein sources needed. This line of investigation also offers a pipeline for evaluating the utility of other novel alternative dietary protein sources for supporting healthy muscle aging. Ultimately, such data suggest increased dietary protein requirements (per serving and RDA) for older adults could be met, at least in part, from mycoprotein, with no evidence to date that this would compromise on protein quality.

**Insect-derived dietary protein sources**

While technically still an animal-derived protein source, insects are not commonly consumed as a protein source (at least in the Western world), but are worth considering as an emerging and viable subcategory for supporting dietary protein provision.63 Over 2000 species of insects are used for human consumption (entomophagy) worldwide. The most commonly consumed insects are: beetles (ie, the order Coleoptera) (31%); caterpillars (Lepidoptera) (18%); sawflies, wasps, bees, and ants (Hymenoptera) (15%); crickets, grasshoppers, and locusts (Orthoptera) (13%); true bugs (Hemiptera) (11%); termites (Isoptera) (3%); dragonflies (Odonata) (3%); flies (Diptera) (2%); and "others" (including cockroaches [Blattodea]) (5%).265 Insects are usually consumed in adult form, but also as the eggs, larvae, and pupae of certain insects. The nutritional composition between and within insect orders is highly variable, but also offers a pipeline for evaluating the utility of other novel alternative dietary protein sources for supporting healthy muscle aging. Ultimately, such data suggest increased dietary protein requirements (per serving and RDA) for older adults could be met, at least in part, from mycoprotein, with no evidence to date that this would compromise on protein quality.

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Ramos-Elorduy et al demonstrated that the protein digestibility of 78 edible insect species ranged between 77% and 98%,\textsuperscript{274} and rat models showed that the protein digestibility of various insect species among several orders ranged between 50% and 92%.\textsuperscript{200,201,275–277} However, direct comparisons of protein digestibility with that of other (animal or plant) protein sources are not available. It is known that insect protein digestibility is often affected by the presence of the insoluble chitin fraction in the exoskeleton\textsuperscript{278} and removal of the exoskeleton enhances insect protein digestibility,\textsuperscript{279} which implies that processed insect proteins would not be less digestible than animal-, or plant-derived proteins. Also, data from other protein sources (eg, casein, mycoprotein) have indicated that a delayed speed of absorption and/or the presence of fiber does not necessarily impair postprandial muscle protein synthetic responses,\textsuperscript{280,281} with some data implying that fiber-derived short-chain fatty acid production may even confer anabolic effects, as alluded to above.\textsuperscript{234,235}

Human experimentation has begun to assess postprandial insect protein handling, and it has been reported that the amino acid bioavailability following mealworm protein isolate ingestion was comparable with that of soy, but lower than that of whey protein.\textsuperscript{282} The latter is likely an artefact of a brief 2-h postprandial period tilting the data towards the more rapidly digested whey protein. Importantly, Hermans et al recently assessed the muscle protein synthetic response of mealworm ingestion compared with that of an isonitrogenous bolus of milk protein in both the rested and exercised muscle of young men.\textsuperscript{283} Similar postprandial amino acid availability and consequent muscle protein synthetic responses were observed between sources, clearly illustrating the viability of mealworms for facilitating muscle anabolism, at least in younger individuals.\textsuperscript{283} One study has also assessed the prolonged muscle adaptive response to resistance training in combination with insect protein supplementation. Though this study reported that daily mealworm supplementation (over 8 wk) did not augment resistance training–induced increases in lean mass or strength compared with no supplementation, protein intake (1.7 g/kg body weight/d) in the control group was likely sufficient to facilitate an optimal adaptive response (ie, above 1.6 g/kg body weight/d\textsuperscript{226}). The minimal types of insect protein investigated and the lack of any data in active older adults clearly represents an area of impactful future research.

**Algal-derived dietary protein sources**

Algae have been part of the human diet for thousands of years in several regions, including South and Middle America, East-Africa, Asia, and Europe.\textsuperscript{285} There is growing global interest in the effects of algal food products on both environmental and human health, with interest in the latter, to date, mostly attributable to the presence in algae of micronutrients and omega fatty acids.\textsuperscript{286,287} However, of interest is whether algae may also represent a promising dietary protein source for human consumption. Algae are photosynthetic eukaryotic organisms predominantly classified by size as microalgae (unicellular) or macroalgae (multicellular). *Chlorella* is the most prevalent genus of microalgae available for human nutrition, with *Chlorella vulgaris* being the specific species utilized. *Spirulina* (*Arthrospira platensis*), technically a cyanobacteria due to being prokaryotic, is another commercialized and widely cultivated species; it is often colloquially referred to as blue-green microalgae because of its color and photosynthetic capacity. Macroalgae are further subdivided according to pigmentation into brown (phylum: Phaeophyceae), green (phylum: Chlorophyta), and red (phylum: Rhodophyta) algae, and are collectively referred to as seaweed. Of these commonly consumed algae, *spirulina* and *chlorella* are generally available as dried dietary supplements (eg, in tablet or powder format), whereas seaweeds are usually consumed as a natural whole-food product (eg, sushi wrap).\textsuperscript{287,288} Currently, algae supplements are being consumed in relatively low doses (eg, 500 mg capsules or 5 g powder boluses) because of the focus on their micronutrient content, and the larger doses required in order to contribute towards protein requirements are not typical. Indeed, it remains unclear whether consuming (multiple) large portions of algae are associated with potential side effects due to excessive micronutrient intake or palatability issues and, as such, whether further food processing optimizing algae for human consumption is required.

*Spirulina* and *chlorella* species are usually cultivated on land in large open ponds (ie, raceway/circular/ unstirred ponds) or closed systems (ie, bioreactors) that mimic natural environmental circumstances, including temperature, light, pH, oxygen, carbon dioxide, and nutrient supplies.\textsuperscript{289} While open pond systems are relatively cheap and easy to maintain, they are prone to contamination and are heavily weather dependent.\textsuperscript{290} Albeit more expensive and requiring greater energy requirements, closed systems are fully environmentally controlled, resulting in more regular and higher yields, thus being far more economical on energy consumption per gram of biomass produced.\textsuperscript{290} Typically, macroalgae are cultivated in open systems, although closed systems involving more controlled cultivation conditions increasing yields are gaining popularity, despite being more expensive than conventional aquaculture.\textsuperscript{291} The complexities of and varying approaches to algae production, and the state of infancy regarding producing it...
as a dietary protein source in the amounts required for humans, makes direct comparisons over sustainability credentials with other sources difficult to make. However, estimated figures collapsed from multiple studies applying various cultivation methods would suggest GHG emissions associated with microalgae production are favorable when compared with those for protein- and amino acid–matched quantities of meat and dairy protein, and comparable with those for mycoprotein and cereal proteins (Figures 2 and 3).234,235,57,60,64–67

It seems likely that the scope for refining production methods of algae as a human protein source produced at scale may offer enormous opportunity.

Commercially available spirulina and chlorella algae species are dried whole-food sources rich in protein (>50% of total mass), roughly equivalent to dried mycoprotein, as well as various protein isolates (Figure 1, see Table S1 in the Supporting Information online).59,60,62,225 Macroalgae generally possess lower protein contents (red: ~23% of total mass; green: ~15%; brown: ~11%), but there are large variations (5%–40%) across species.61,292 What is perhaps most intriguing about algae is the attractive amino acid profile it possesses. Both micro- (>40% of total protein) and macro-algae (~40%) contain an essential amino acid content in excess of the WHO/FAO/UNU requirements (27.7%) and of the vast majority of plant-based protein sources, broadly analogous to animal-derived proteins (Figure 5).59,61,62,143 Moreover, levels of the individual (key) essential amino acids in algae all appear to meet requirements,61,62,143 with leucine in particular worthy of mention (~9%) (Figure 5).62

In vitro work has shown that protein digestibility (in vivo ileal digestibility unknown) of spirulina and chlorella is approximately 80% and approximately 75%, respectively,62 which is lower than that of skimmed milk (95%), soybean (90%), and egg (94%).62,293 This implies a somewhat lower bioavailability and PDCAAS/DIAAS score for algae when compared with animal-derived protein sources, which is possibly attributable to the rigid structure of the cellullosic and bacterial cell wall for chlorella and spirulina, respectively.286 To some extent, commercialization of algae supplements has attempted to obviate these issues by applying various post-harvesting treatments to ostensibly disrupt the cell wall in order to improve protein digestibility,294 though how successful this is remains to be established. It is also possible, in line with previous discussions around plant, mycoprotein and insects, that the cell wall also contains potentially bioactive factors (e.g., fiber) that may modulate the anabolic response.234,235 No human work is available to date investigating algae protein ingestion and postprandial amino acid handling, and so data on in vivo protein digestion, amino acid absorption and bioavailability, and muscle protein turnover are absent. Encouraging signs can be found in various in vitro and animal studies, which report that algal proteins are capable of stimulating muscle protein synthesis,295 increasing muscle strength,296 and mitigating age-related muscle atrophy.297,298 Human work analogous to that carried out for other dietary protein sources is now required, investigating various micro- and macro-algae species, cultivated and processed in various manners, and their ability to support muscle protein synthesis and adaptive responses in active (older) adults, with particular emphasis on how algae can be incorporated into the human food chain.

Synthetic “meats”

The first laboratory-grown burger was launched in 2013 to great media and scientific fanfare,299,300 the result of the relatively new food technology concept of in vitro–cultured “meat.” It involves the production of edible biomass from cell culture, generally of stem cells harvested from the muscle of live animals.300 Technically, if not necessarily philosophically, being “meat,” such an approach has clear ethical and environmental advantages over conventional livestock agriculture: less land usage and GHG emissions, considered safer (lower contamination risk), securer and more efficient (high-yield and quicker production process).301 However, the cultivation of laboratory-grown meat is associated with relatively greater energy requirements compared with those of non-animal-derived protein sources, although similar energy requirements are reported for beef.302 At present, however, several challenges lie ahead in refining and optimizing in vitro meat production processes to produce at scale. Optimal sources of stem cells and culture media ingredients are yet to be refined, and high production costs are the major scientific and economic initial barriers to successful commercialization.301 In addition, mixed findings have been reported regarding potential consumer acceptance due to perceptions of “unnaturalness” increasing feelings of “disgust.”303–305 Synthetic meats hold great future promise as a further category of dietary protein for healthy aging, but significant barriers first need to be overcome.

CONCLUSION

Contemporary and imminent management of age-related sarcopenia will be predicated on modifiable lifestyle factors: optimized physical activity, and optimized dietary protein intake (through promotion of a developed and refined RDA). The rapidly growing worldwide aging population dictates the increasingly consequential stakes for public health and global
economies. Animal-derived proteins provide the robust evidence base used to derive dietary protein recommendations for supporting healthy aging. However, this reliance is coming into conflict with government and societal concerns surrounding whether the environmental burdens associated with continuing to increase animal protein production and consumption is commensurate with a sustainable food future. While the shift towards alternative dietary proteins is seemingly desirable and gaining momentum, consideration as to what may (or may not) be viable (or optimal) sources needs to keep pace. Of importance is clarity as to what constitutes a “high quality” dietary protein source, especially in the context of older demographics, who have a lower anabolic sensitivity to protein intake. Further, what is sufficient or optimal from the perspective of muscle anabolism in older adults must be considered alongside potentially compromised appetite, energy intake, and metabolic health, amid other concerns.

Human data are beginning to accumulate that assess aspects of nutritional physiology relating to protein digestibility, postprandial metabolism, and muscle adaptive responses consequent to ingesting plant-, fungal-, and insect-derived protein alternatives. Such data are beginning to shed light on how future recommendations around dietary protein to support healthy aging could incorporate such protein sources (eg, blended plant proteins, mycoprotein, or mealworms). While equivalent data around algal-derived protein sources are not yet available, algae promise to be an attractive additional source of dietary protein into the future. Accumulation of data in this space will allow: (a) a comprehensive research portfolio of available and viable protein sources across all (sub)categories; (b) close the loop with respect to having analogous data for all sources, spanning nutritional composition through to mechanistic metabolism (ie, Figure 4) and functional outcomes across the lifespan, all of which will together be necessary to provide a tool to enable global policy change.

Finally, it is greatly underappreciated how establishing a sustainable future for dietary protein to support healthy aging must be underpinned by multidisciplinary research and cooperation between research communities and various other stakeholders and societal sectors. Indeed, the production and consumption of food is a complex and ubiquitous issue that intersects health, culture, economics, and environmentalism. The following is is a non-exhaustive list of what will be required in order to solve this very modern issue, insofar as it can be “solved”: bioscientists refining dietary protein production methods, environmental scientists accumulating sustainability data, mathematical modelers predicting economic impacts and the viability of changing supply, physiologists interrogating the impact of the various dietary proteins on human metabolism, social scientists looking at behavioral change and food acceptability/choices; food scientists and industries looking at commercialization and product development; public health officials, epidemiologists, and clinicians monitoring changing nutritional habits; and government agencies enacting policy change. It is crucial that we respond optimally to the issue of sustainable dietary protein—an issue that appears to be increasingly permeating all realms of discussion of the future of nutrition for healthier aging.

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Figures. Figure 4 was created with BioRender.com

Supporting Information

Table S1 Protein content and greenhouse gas emissions of various commercially available whole-food sources

Table S2 Essential amino acid composition of various commercially available whole-food sources

Table S3 Non-essential amino acid composition of various commercially available whole-food sources

Figure S1 Essential amino acid content (mean ± SEM) expressed in percentage of total protein of histidine (A), isoleucine (B), leucine (C), lysine (D), methionine (E), phenylalanine (F), threonine (G), tryptophan (H), and valine (I) of various categories of dietary protein sources

Figure S2 Non-essential amino acid content (mean ± SEM) expressed in percentage of total protein of alanine (A), arginine (B), aspartic acid (C), cysteine (D), glutamic acid (E), glycine (F), proline (G), serine (H), threonine (I), and tyrosine (J) of various categories of dietary protein sources.
(D), glutamic acid (E), glycine (F), proline (G), serine (H), and tyrosine (I) of various categories of dietary protein sources

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