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Characterising the muscle anabolic potential of dairy, meat and plant-based protein sources in older adults

Stefan H. M. Gorissen[1]* and Oliver C. Witard[2]
[1]*Exercise Metabolism Research Group, Department of Kinesiology, McMaster University, Canada*
[2]*Physiology, Exercise and Nutrition Research Group, Faculty of Health Sciences and Sport, University of Stirling, Scotland, FK9 4LA, UK*

The age-related loss of skeletal muscle mass and function is caused, at least in part, by a reduced muscle protein synthetic response to protein ingestion. The magnitude and duration of the postprandial muscle protein synthetic response to ingested protein is dependent on the quantity and quality of the protein consumed. This review characterises the anabolic properties of animal-derived and plant-based dietary protein sources in older adults. While approximately 60 % of dietary protein consumed worldwide is derived from plant sources, plant-based proteins generally exhibit lower digestibility, lower leucine content and deficiencies in certain essential amino acids such as lysine and methionine, which compromise the availability of a complete amino acid profile required for muscle protein synthesis. Based on currently available scientific evidence, animal-derived proteins may be considered more anabolic than plant-based protein sources. However, the production and consumption of animal-derived protein sources is associated with higher greenhouse gas emissions, while plant-based protein sources may be considered more environmentally sustainable. Theoretically, the lower anabolic capacity of plant-based proteins can be compensated for by ingesting a greater dose of protein or by combining various plant-based proteins to provide a more favourable amino acid profile. In addition, leucine co-ingestion can further augment the postprandial muscle protein synthetic response. Finally, prior exercise or n-3 fatty acid supplementation have been shown to sensitise skeletal muscle to the anabolic properties of dietary protein. Applying one or more of these strategies may support the maintenance of muscle mass with ageing when diets rich in plant-based protein are consumed.

Plant-based protein source: Animal-derived protein source: Muscle protein synthesis: Healthy musculoskeletal ageing

Ageing is accompanied by a decline in muscle mass and function, termed sarcopenia(1). Sarcopenia increases the risk for falls and fractures, dependence, morbidity and mortality(2). The underlying cause of sarcopenia is multifactorial and complex in nature. Contributing factors include, but are not limited to, reduced physical activity levels, poor diet, chronic low-grade systemic inflammation, elevated levels of oxidative stress, mitochondrial dysfunction and hormonal changes(3–5). Sarcopenia imposes significant burden on healthcare systems. In 2000, the estimated annual healthcare cost of sarcopenia in the USA reached $18·5 billion, representing 1·5 % of total healthcare expenditures for that year(6). In order to treat or prevent sarcopenia, nutritional strategies must be developed to help increase or maintain skeletal muscle mass with advancing age.

Skeletal muscle mass is regulated by the balance between muscle protein synthesis and muscle protein breakdown(7). Loss of muscle mass results from a negative net muscle protein balance, i.e. when muscle protein

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***Corresponding author:** Dr S. Gorissen, email gorisses@mcmaster.ca

breakdown exceeds muscle protein synthesis over a given period of time. Muscle protein synthesis and muscle protein breakdown are concurrent and constant processes that are highly responsive to physical activity and protein intake(8). Muscle protein synthesis is more responsive to both stimuli than muscle protein breakdown(9). Thus, changes in muscle protein synthesis are primarily responsible for changes in muscle mass in response to exercise and nutrition, at least in healthy individuals(10). Dietary protein provides amino acids that can be used as precursors (i.e. building blocks) for muscle protein synthesis. Moreover, the essential amino acid leucine is not only a building block for muscle protein synthesis, it acts as a signalling molecule that can directly activate the muscle protein synthetic machinery. Several studies have compared the postprandial muscle protein synthetic response to protein intake between young and older individuals(11,12). Some studies observed lower postprandial muscle protein synthesis rates in older adults compared with the young, which has resulted in a concept termed anabolic resistance(13). Not all studies have been able to detect anabolic resistance, which might be related to the limited number of participants who are generally included in these financially expensive tracer studies(14). However, a more comprehensive evaluation of the muscle protein synthetic response to protein intake between young and older individuals was recently conducted by Wall *et al.*(14), whereby data were pooled from multiple studies conducted within the same laboratory using an almost identical study design. Study findings revealed a markedly reduced muscle protein synthetic response to the ingestion of a single meal-like 20 g bolus of casein in older compared with young adults(14). These comprehensive data support the existence of anabolic resistance with ageing.

The aetiology of anabolic resistance with ageing is not entirely understood, but is proposed to be mediated by impairments in several physiological processes(13). A reduced rate of dietary protein digestion and amino acid absorption and/or a greater splanchnic amino acid retention may limit the postprandial availability of amino acids for muscle protein synthesis(15,16). In addition, a decline in insulin-mediated capillary recruitment, muscle tissue perfusion and the abundance or functionality of amino acid transporters may limit the delivery of amino acids to the muscle and the uptake of amino acids by the muscle(17–19). At the molecular level, an impaired activation of mechanistic target of rapamycin complex 1 and downstream signalling (e.g. p70S6 kinase, 4E-BP1) that regulates muscle protein synthesis also may contribute to anabolic resistance with ageing(20,21). Understanding the relative contribution of these processes to anabolic esistance with ageing is of critical importance for the design of effective nutritional strategies for combatting sarcopenia. Several factors are known to influence the muscle protein synthetic response to protein ingestion, most notably the quantity of protein consumed on a meal-by-meal basis(22). In addition, the quality (i.e. source) of ingested protein has been shown to modulate the postprandial muscle protein synthetic response(23). Accordingly, the primary focus of this review is to compare the muscle anabolic capacity of animal-derived (dairy and meat-based) proteins with various plant-based proteins in older adults.

Anabolic properties of animal-derived protein sources

Several studies have demonstrated that animal-derived protein sources such as dairy (e.g. milk and eggs) and meat (e.g. beef) elicit a robust stimulation of muscle protein synthesis in older adults(23). However, not all animal-based protein sources are comparable in terms of anabolic properties that determine the amplitude and duration of the postprandial muscle protein synthetic response. For example, whey protein is characterised as a fast protein based on its rapid protein digestion and amino acid absorption kinetics, whereas casein clots in the stomach and is slowly digested and absorbed(24,25). The ingestion of 20 g fast digestible whey protein, that is particularly high in leucine content, has been shown to stimulate muscle protein synthesis to a greater extent compared with a matched dose of slowly digestible micellar casein in older men(26). These findings are consistent with similar studies in young adults(27) and highlight the importance of a rapid rise in blood leucine concentrations for stimulating a robust increase in muscle protein synthesis(28).

Although whey protein has consistently been shown to elicit a robust stimulation of muscle protein synthesis, whey protein represents a fraction of milk and is commonly co-ingested with casein(29). Accordingly, a recent study assessed the postprandial muscle protein synthetic response to ingesting 20 g whey protein compared with a milk protein concentrate composed of both whey protein and casein(29). Study findings revealed a more rapid appearance of circulating amino acids after whey protein ingestion; however there was no difference in the postprandial muscle protein synthetic response between whey protein and milk protein concentrate in middle-aged men. In terms of comparing the anabolic potential of animal-derived protein-rich foods, we recently assessed postprandial protein handling and the subsequent muscle protein synthetic response to the ingestion of 350 ml fluid skimmed milk compared with 160 g cooked lean minced beef (both providing 30 g protein) during recovery from resistance exercise in young men(30). Beef was more rapidly digested and absorbed, which resulted in a greater rise in plasma amino acid availability and higher peak plasma leucine concentrations. Skimmed milk ingestion resulted in a moderate but rapid rise in circulating plasma leucine and stimulated muscle protein synthesis to a greater extent during the early 0–2 h recovery period than beef. Taken together, these data suggest that milk is equally effective as whey protein and superior to beef with regard to stimulating muscle protein synthesis.

Food matrix and texture may represent another important factor that modulates the muscle protein synthetic response to protein ingestion in older adults(23). A recent study demonstrated that minced beef is more rapidly digested and absorbed than beef steak, resulting in a greater availability of protein-derived amino acids in the circulation and a more positive whole-body net protein balance after the ingestion of minced beef compared with beef steak(31). The 135 g portion of beef administered in this study provided about 20 g protein, which was unable to stimulate muscle protein synthesis. Consistent with this observation, a previous dose–response study demonstrated that 113 g minced beef (24 g protein) was not sufficient to stimulate muscle protein synthesis under both rested and post-exercise conditions in middle-aged men(32). Instead, 170 g beef, providing 36 g protein, was required to stimulate muscle protein synthesis. Taken together, these data suggest that minced beef may be more effective in stimulating muscle protein synthesis compared with beef steak at higher doses of protein intake.

Global differences in food sources constituting daily protein intake

To date, most studies in older adults that have assessed the postprandial muscle protein synthetic response to protein intake have administered an animal-derived protein source(26,31,33–37). Relatively few studies have characterised the muscle protein synthetic response to ingestion of a plant-based protein source(10,38,39). This gap in knowledge may be considered surprising given that a greater variety of plant-based protein-rich foods are readily available compared with animal-derived protein foods and most dietary protein consumed worldwide is in fact derived from plant (60 %) rather than animal (40 %) sources (Table 1)(40). An estimated 4 billion people live primarily on a plant-based diet, while an estimated 2 billion people worldwide live primarily on a meat-based diet(41). Of the plant-based food sources, cereals provide the greatest contribution, responsible for approximately 65 % of plant protein intake and 40 % of total protein intake. Pulses, nuts, seeds and vegetables provide a moderate contribution (2·2–10·4 g/d) to daily protein intake, whereas lower amounts of protein are provided by potatoes and fruit (about 3 g/d). Importantly, the contribution of plant and animal-based protein sources to total daily protein intake is specific to the continent or country of interest. In Africa and Asia, plant-based foods provide 77 and 66 % of total protein intake, respectively, whereas the contribution of animal-derived food sources to total dietary protein intake is greater in the USA (56 %), Europe (57 %) and Oceania (65 %). Across the USA, Europe and Oceania, meat and dairy sources provide the greatest contribution (about 80 %) to daily animal protein intake, whereas as little as 7 g meat and 4 g milk is consumed *per capita* per day in Africa. It may be argued that future research designed to assess the muscle protein synthetic response to an ingested protein source should focus on the most commonly consumed protein source in any given country.

Sustainability of commonly consumed dietary protein sources

A more advanced understanding of the anabolic potential of various plant-based protein sources also may be considered critical given concerns regarding the global sustainability of animal-based protein diets. A sustainable diet may be defined as ‘a diet with low environmental impact that contributes to food and nutrition security and to healthy life for present and future generations’(42). The food supply chain accounts for about 20 % of all annual greenhouse gas emissions attributed to the UK(43). In the UK, the consumption of animal-derived protein foods is increasing at a rate 2-fold greater than plant-based protein foods(44). Diets that primarily contain animal-derived food sources (e.g. meat and dairy products) are associated with high greenhouse gas emissions (>4 kg carbon dioxide equivalents (CO_2e)/kg edible weight; Fig. 1)(42). According to recent UK estimates, the production and consumption of beef (about 70 kg CO_2e/kg) and pork (8 kg CO_2e/kg edible weight) contributes most of all food sources to greenhouse gas emissions(45). Interestingly, unlike most other dairy products (e.g. cheese and eggs), milk is associated with only moderate greenhouse gas emissions. The production and consumption of most plant-based protein foods, including wheat, oat and potato are associated with low greenhouse gas emissions (<1 kg CO_2e/kg edible weight)(42). Notable exceptions include rice (4 kg CO_2e/kg edible weight) and to a lesser extent soya (2 kg CO_2e/kg edible weight)(45). Therefore, on a gram-for-gram basis, commonly consumed plant-based protein-rich foods are, for the most part, considered to be more environmentally sustainable compared with animal-derived proteins, especially from meat sources(41). However, as a note of caution, it is widely appreciated that recommendations for improving protein-based food choices to reduce greenhouse gas emissions must be balanced against protein recommendations for improving health(42,44), of which the maintenance of muscle mass becomes increasingly important with advancing age(46). It follows that a critically important topic in the field of protein nutrition for healthy musculoskeletal ageing includes comparing the anabolic capacity of plant and animal-based proteins for combatting sarcopenia.

Anabolic properties of plant-based protein sources

Soya protein is one of the few plant-based proteins that has been studied for its muscle anabolic potential in human subjects. Wilkinson *et al.*(47) compared the anabolic response to consuming soya and milk within a mixed macronutrient beverage containing 18 g protein. The consumption of milk after a single bout of resistance exercise increased postprandial muscle protein synthesis rates to a greater extent compared with the ingestion of soya in healthy young men(47). The authors speculated that due to the more rapid digestion of soya protein and therefore faster and greater delivery of amino acids from the gut to the liver, more of the soya protein-

Table 1. Sources of dietary protein intake

	World	Africa	Asia	Americas	Europe	Oceania	Canada	Netherlands	UK
Total	81·2	69·1	77·6	93·3	102·1	101·6	105·0	111·7	103·2
Animal	32·1	16·1	26·6	52·1	57·9	66·2	54·7	75·8	58·3
Meat	14·5	7·2	10·7	29·3	26·3	36·2	30·8	35·1	29·3
Poultry	5·2	2·4	3·3	13·2	8·9	14·9	13·1	9·9	12·8
Pork	4·7	0·4	4·7	4·8	9·6	5·4	6·2	10·9	7·1
Beef	3·5	2·5	1·7	10·8	5·8	11·5	11·2	7·1	6·5
Milk	8·2	4·0	5·8	14·5	19·1	17·0	12·6	28·9	19·1
Fish, seafood	5·2	3·1	5·8	3·6	6·6	6·8	5·7	6·9	5·5
Eggs	2·8	0·8	2·9	3·4	4·0	2·5	3·8	4·4	3·4
Offal	1·1	0·9	1·0	1·1	1·7	3·5	0·4	0·4	0·9
Plant	49·1	53·0	50·9	41·1	44·2	35·4	50·3	36·0	44·9
Cereals	31·8	33·8	33·2	25·2	30·1	21·7	26·3	21·7	28·0
Wheat	15·9	11·3	15·7	13·6	25·7	17·9	20·5	17·9	24·3
Rice	10·1	4·7	14·5	3·7	0·9	2·4	2·5	0·5	1·3
Maize	3·6	9·9	1·8	7·0	1·3	0·8	2·5	0·4	0·6
Oats	0·1	0·0	0·0	0·4	0·5	0·2	0·2	0·4	1·6
Pulses, nuts and seeds	7·5	10·4	7·5	8·2	3·2	4·0	13·0	3·4	4·0
Soyabeans	1·4	0·9	1·8	0·7	0·2	0·1	0·9	0·0	0·0
Peas	0·5	0·4	0·5	0·5	0·7	0·5	1·0	0·6	1·2
Vegetables	4·9	2·2	6·4	2·2	3·6	3·3	3·3	3·0	3·3
Starchy roots	2·3	4·1	1·7	2·1	3·5	2·6	3·2	4·0	4·2
Potatoes	1·5	0·8	1·3	1·7	3·5	2·1	3·1	4·0	4·2
Fruits	1·1	1·2	1·0	1·4	1·3	1·2	1·5	1·9	1·6

Data are expressed as g protein *per capita* per d. Food sources shown in table provide at least 96 % of daily protein intake. Data are derived from Statistics Division of FAO of the UN, Food Balance Sheets 2013(40).

derived amino acids were directed towards urea production and serum protein synthesis(48), rather than muscle protein synthesis(47). Milk ingestion resulted in a moderate but sustained rise in plasma amino acid concentrations and subsequently a more prolonged positive net protein balance across the leg(47). Consistent with this observation, the chronic consumption of milk immediately and 1 h after each exercise session (providing 35 g protein) during 12 weeks resistance training resulted in greater gains in lean body mass when compared with the consumption of isonitrogenous amounts of soya(49). Thus, milk has been shown to be more effective in stimulating muscle protein synthesis compared with a soya-based protein beverage in resistance trained young men.

The postprandial muscle protein synthetic response to the ingestion of isolated soya protein also has been compared with the constituent milk proteins, whey and casein, in young men under resting conditions(27). The ingestion of soya protein stimulated muscle protein synthesis to a greater extent when compared with casein, but the postprandial muscle protein synthetic response to soya protein tended to be lower when compared with whey protein. This divergent muscle protein synthetic response was likely attributed to differences in protein digestion kinetics and leucine content. Whey protein is rapidly digested and exhibits a high leucine content (2·3 g), whereas soya protein is rapidly digested but exhibits a lower leucine content (1·8 g) and casein is slowly digested and exhibits a lower leucine content (1·8 g). As such, the ingestion of whey protein, soya protein and casein results in a high, medium and low rise in plasma leucine concentrations, respectively, which is reflected by the magnitude of the postprandial increase in muscle protein synthesis rates(27). Studies in older individuals have shown that postprandial muscle protein synthesis rates after the ingestion of 24 g soya protein are lower when compared with an equal amount of protein provided by beef(39). Consistent with this observation, the muscle protein synthetic response to graded intakes of soya protein also was shown to be lower when compared with whey protein in older adults(10). In fact, ingesting up to 40 g soya protein failed to substantially elevate muscle protein synthesis rates from basal, fasting rates(10). Thus, the ingestion of soya protein may stimulate muscle protein synthesis in young individuals, albeit to a lesser extent compared with some animal-derived proteins (e.g. whey). However, based on current evidence, soya protein intake is unable to stimulate muscle protein synthesis in older adults, at least at the doses (up to 40 g) investigated to date.

Wheat protein is the most abundant plant-based dietary protein source worldwide comprising approximately 20 % of total protein intake (Table 1)(40). We recently assessed the anabolic properties of wheat protein when compared with casein and whey protein in healthy older men(38). We provided 35 g whey, casein, or wheat protein containing 4·4, 3·2 or 2·5 g leucine, respectively, which is suggested to be sufficient to activate the muscle protein synthetic machinery(50). The ingestion of whey protein increased postprandial plasma leucine concentrations to a greater extent compared with casein and wheat protein. The ingestion of casein and wheat protein resulted in a similar peak in plasma leucine concentrations, with a more sustained elevation of plasma leucine concentrations after casein

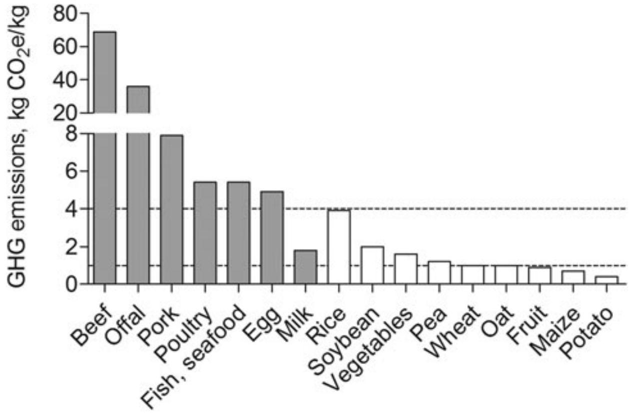

Fig. 1. Estimated greenhouse gas (GHG) emissions (kg CO_2e/kg edible weight) for the most common animal-based (grey bars) and plant-based (white bars) dietary protein sources. Low GHG emissions, <1·0 kg CO_2e/kg edible weight; Medium GHG emissions, 1·0–4·0 kg CO_2e/kg edible weight; High GHG emissions, >4·0 kg CO_2e/kg edible weight; CO_2e, carbon dioxide equivalent. Estimated values are based on UK data derived from Scarborough *et al.*(45).

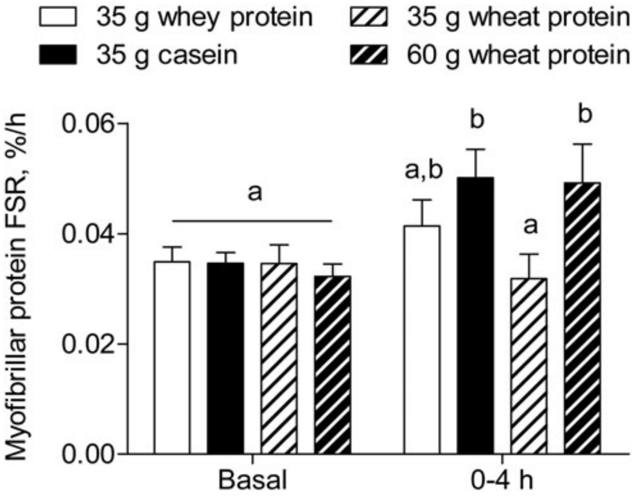

Fig. 2. Myofibrillar protein fractional synthetic rate (FSR, %/h) during the fasting state (Basal) and over the 4 h postprandial period after the ingestion of 35 g whey protein, 35 g casein, 35 g wheat protein, or 60 g wheat protein in healthy older men. Values are means ± SEM, *n* 12/group. Labelled bars without a common letter differ, $P < 0{\cdot}05$. Data are derived from Gorissen *et al.*(38).

ingestion. Interestingly, only the ingestion of casein stimulated muscle protein synthesis and the postprandial muscle protein synthetic response to the ingestion of wheat protein was significantly lower when compared with casein (Fig. 2). Ingesting a greater amount of wheat protein (i.e. 60 g), matched for the leucine content of whey protein, prolonged the postprandial increase in plasma leucine concentrations and increased muscle protein synthesis rates to a similar extent as casein(38). These data demonstrate that a larger amount of wheat protein compared with casein is required to stimulate muscle protein synthesis in older men.

The assumption that plant-based proteins exhibit inferior muscle anabolic potential compared with animal proteins is essentially based on data obtained from acute metabolic studies that assessed the postprandial muscle protein synthetic response to ingested soya and wheat protein(10,27,38,39,47,49). An explanation for the reduced postprandial muscle protein synthetic response after the ingestion of soya or wheat may relate, at least in part, to the lower digestibility of these plant proteins compared with animal proteins. Animal-based protein sources such as dairy, meat and fish are highly digestible with digestibility scores exceeding 90 %. In contrast, plant-based protein sources such as rice, wheat, soya and potato exhibit lower digestibility scores ranging from 45 to 80 %(51). As such, less of the dietary protein contained in a plant source is absorbed by the small intestine, resulting in a lower availability of dietary protein-derived amino acids for muscle protein synthesis. However, after removal of anti-nutritional factors that interfere with protein digestion and absorption, purified plant-based proteins are likely to exhibit digestibility scores similar to animal-derived proteins. Moreover, the essential amino acid composition of plant-based proteins may be suboptimal for the stimulation of muscle protein synthesis compared with animal-derived proteins. If an (essential) amino acid is limiting, protein synthesis is compromised and all other amino acids will be oxidised rather than utilised for protein synthesis(52). Plant-based proteins generally have an inadequate lysine and/or methionine content (Table 2)(53). For example, wheat protein contains low amounts of lysine and methionine, both below the amino acid requirement as defined by the WHO/FAO/UNU(52). Maize, rice and oat protein are low in lysine, whereas soyabean and pea protein are low in methionine. However, potato and quinoa protein contain sufficient amounts of all essential amino acids. As such, the assertion that all plant-based protein sources exhibit inferior muscle anabolic potential may be considered somewhat premature. Future studies are warranted to assess whether potato or quinoa protein have the capacity to stimulate muscle protein synthesis in older individuals.

Overcoming the perceived inferior anabolic properties of plant-based proteins

Dose of protein

Protein dose–response studies have been conducted to assess how much protein is required to maximally stimulate muscle protein synthesis. We recently assessed the postprandial muscle protein synthetic response to whey protein intakes ranging from 0 to 40 g in young men(54). The results indicated that a maximal stimulation of muscle protein synthesis was achieved after ingesting 20 g whey protein. A similar study in older men showed a dose–response relationship up to 40 g whey protein(37), indicating that older individuals require a higher protein dose to maximally stimulate muscle protein synthesis. To date, only one study has assessed the postprandial muscle protein synthetic response to graded intakes of plant-based protein(10). The ingestion of up to 40 g soya protein (3·2 g leucine) was unable to induce a measurable increase in muscle protein synthesis rates

Table 2. Amino acid composition of various plant-based and animal-derived proteins

	Plant sources								Animal sources								Amino acid requirements
	Wheat	Maize	Rice	Oats	Soyabean	Pea	Potato	Quinoa	Whey	Milk	Casein	Beef	Pork	Chicken	Egg	Cod	
Essential amino acids																	
Histidine	2·1	2·8	2·5	2·3	2·6	2·5	2·0	3·1	1·9	2·7	2·7	3·6	2·6	2·9	2·4	2·8	1·5
Isoleucine	4·1	3·8	3·8	4·1	4·7	4·6	4·9	4·7	6·4	5·1	5·0	5·0	5·4	5·9	6·2	4·5	3·0
Leucine	6·8	12·9	8·2	7·9	8·0	7·4	7·8	7·8	9·9	9·5	8·9	8·5	8·5	8·2	8·7	8·2	5·9
Lysine	1·4	2·8	3·8	4·0	6·6	8·2	6·2	7·2	9·2	6·9	7·6	9·3	9·4	8·8	6·9	9·7	4·5
Methionine	1·6	2·0	2·3	1·8	1·3	1·0	1·7	2·6	2·0	2·5	2·6	2·8	2·8	2·8	3·3	3·3	1·6
Phenylalanine	5·1	5·0	5·2	5·4	5·1	5·0	5·2	5·3	3·8	4·6	4·9	4·6	4·4	4·4	5·6	4·9	3·8
Threonine	2·5	3·7	3·9	3·6	4·0	4·4	4·9	4·5	6·7	4·0	4·3	4·8	4·8	4·4	5·0	5·0	2·3
Valine	4·2	5·0	5·5	5·5	4·9	5·1	6·1	5·8	6·3	6·2	6·3	5·2	5·9	5·7	6·7	5·1	3·9
Total EAA	27·8	38·1	35·2	34·7	37·1	38·2	38·8	40·9	46·2	41·6	42·4	43·7	43·8	43·2	44·8	43·5	27·7
Non-essential amino acids																	
Alanine	2·5	7·8	6·0	4·9	4·4	4·4	5·8	6·1	4·8	3·3	2·9	6·1	6·0	3·8	5·8	6·5	
Arginine	3·0	4·3	8·3	6·8	7·4	10·3	6·5	9·1	2·5	3·3	3·5	6·6	6·5	6·2	6·0	6·4	
Aspartic acid	3·0	6·5	10·3	8·4	12·0	11·9	16·1	9·4	10·2	7·5	6·7	9·4	9·7	10·2	9·5	10·3	
Cystine	2·1	1·6	1·1	2·9	1·4	1·2	0·8	0·0	1·7	0·9	0·3	1·3	1·3	1·5	2·4	1·1	
Glutamic acid	36·9	19·6	20·6	22·8	19·2	17·5	13·3	15·4	17·8	20·0	20·6	15·9	15·8	16·7	12·5	15·3	
Glycine	3·1	3·8	5·0	5·1	4·3	4·4	4·9	6·7	2·2	1·9	1·8	5·1	4·7	5·9	3·3	4·5	
Proline	13·0	9·2	4·7	5·6	5·6	4·2	4·9	4·0	6·3	11·3	10·8	3·9	4·2	4·6	4·1	3·8	
Serine	4·9	5·1	5·4	5·1	5·3	4·7	5·4	4·8	5·2	5·5	5·6	4·2	4·2	4·3	7·5	4·8	
Tyrosine	3·6	4·0	3·5	3·6	3·2	3·0	3·6	3·6	3·0	4·8	5·4	3·8	3·7	3·7	4·1	3·8	
Total NEAA	72·2	61·9	64·8	65·3	62·9	61·8	61·2	59·1	53·8	58·4	57·6	56·3	56·2	56·8	55·2	56·5	

Data are expressed as % of total protein. EAA, essential amino acids; NEAA, non-essential amino acids. Data are derived from FAO Nutritional Studies(88). Amino acid requirements for adults (far right column) are derived from WHO/FAO/UNU(52).

under resting conditions in older individuals(10), suggesting that even greater amounts of plant-based protein are required to stimulate muscle protein synthesis in older adults. With a view to overcoming the inferior anabolic capacity of wheat protein, we recently provided older men with a 60 g bolus of wheat protein constituting 4·4 g leucine(38). The ingestion of this high dose of wheat protein effectively stimulated muscle protein synthesis to a similar extent as dairy protein. Interestingly, ingesting the higher dose of wheat protein did not further increase the amplitude of peak plasma leucine concentrations compared with the lower 35 g dose of wheat protein, but prolonged the postprandial elevation in plasma leucine (and total essential amino acid) concentrations(38). These data suggest that the kinetics of amino acid appearance in the blood rather than the absolute increase in leucine or essential amino acid concentrations determine the postprandial muscle protein synthetic response. Moreover, essential amino acids should be made available for muscle protein synthesis in older adults during the late postprandial period. Although effective, the ingestion of (very) high doses of plant-based protein may not represent a practical strategy for stimulating muscle protein synthesis in older adults with low appetite. In addition, it could be questioned whether plant-based proteins are still more environmentally sustainable when accounting for the greater dose of ingested protein necessary to maximally stimulate muscle protein synthesis. Therefore, more practical, cost-effective and sustainable strategies are discussed later.

Protein blends

The use of protein blends recently has received considerable attention as a possible strategy to overcome the inferior anabolic properties of plant-based proteins(53). Combining two or more plant-based protein sources may potentially overcome any deficiencies in a single essential amino acid that may be prevalent if a single plant protein is consumed. Plant-based proteins are generally low in lysine and/or methionine content when compared with animal-based proteins, which may compromise the postprandial muscle protein synthetic response(53). As described earlier, many plant-based proteins are low in either lysine or methionine, but contain ample amounts of the other essential amino acids (Table 2). Theoretically, combining a plant-based protein, which is low in lysine but high in methionine with a plant-based protein, which is low in methionine but high in lysine will result in a protein blend that contains sufficient amounts of all essential amino acids required for stimulating muscle protein synthesis. For example, maize, rice and oat protein are low in lysine but high in methionine, whereas soyabean and pea protein are low in methionine but high in lysine. Accordingly, we designed three protein blends that combine two plant-based proteins in a 50/50 ratio: maize/soyabean, rice/soyabean and rice/pea. The lysine and methionine contents of all the three blends exceed the amino acid requirement as defined by the WHO/FAO/UNU (4·5 and 1·6 %, respectively(52); Fig. 3). Multiple other protein blends can be formulated that combine two or more complementary plant-based proteins at different ratios in order to provide sufficient amounts of all nine essential amino acids. However, it remains to be determined whether these protein blends have the capacity to stimulate muscle protein synthesis to a similar extent as dairy or meat-based proteins.

Alternatively, combining plant-based with animal-derived proteins may result in a protein blend that will capitalise on the unique digestive properties of each type of protein, allowing for an optimal blood availability of amino acids to increase the amplitude and duration of the postprandial muscle protein synthetic response. A series of studies have been conducted using a plant/animal protein blend composed of 50 % caseinate, 25 % whey protein and 25 % soya protein(55–58). In young adults, ingesting 19 g of this protein blend after a single bout of resistance exercise increased muscle protein synthesis rates and resulted in a more sustained elevation of muscle protein synthesis when compared with the ingestion of a leucine-matched amount of whey protein(58). In terms of translating these acute data in young adults to a chronic setting, dietary supplementation with this protein blend during 12 weeks resistance training tended to enhance gains in lean body mass compared with placebo in young men (2·9 *v*. 2·0 kg, respectively), whereas no further increase in lean body mass was observed after training with whey protein supplementation (2·3 kg)(56). In older adults, ingesting a higher 30 g dose of the protein blend during recovery from resistance exercise failed to stimulate muscle protein synthesis above basal resting values, whereas whey protein ingestion did stimulate muscle protein synthesis during the early and entire post-exercise recovery period(55). The absence of a measureable increase in muscle protein synthesis after ingesting the protein blend is surprising since it has consistently been shown that prior exercise sensitises skeletal muscle to the anabolic properties of dietary protein(59,60). It seems likely that the higher basal resting muscle protein synthesis rates in the protein blend condition precluded the ability to detect a significant stimulation of muscle protein synthesis. Nonetheless, absolute values of postprandial muscle protein synthesis rates after the ingestion of the protein blend were similar when compared with whey protein. These data suggest that a plant/animal protein blend can stimulate muscle protein synthesis to a similar extent as whey protein. However, the addition of a small amount of soya protein to milk protein does not substantially reduce dairy protein intake. Moreover, this protein blend does not seem to offer benefits regarding sustainability as milk is relatively environmentally sustainable compared with other animal-based protein sources. Future studies should identify other plant/animal protein blends with a higher plant-based protein content that could serve as a more sustainable dietary protein source to preserve muscle mass in the ageing population.

Leucine co-ingestion

Accumulating evidence suggests that the strongest independent predictor of muscle anabolic potential is the leucine content of the ingested protein source(61). In addition

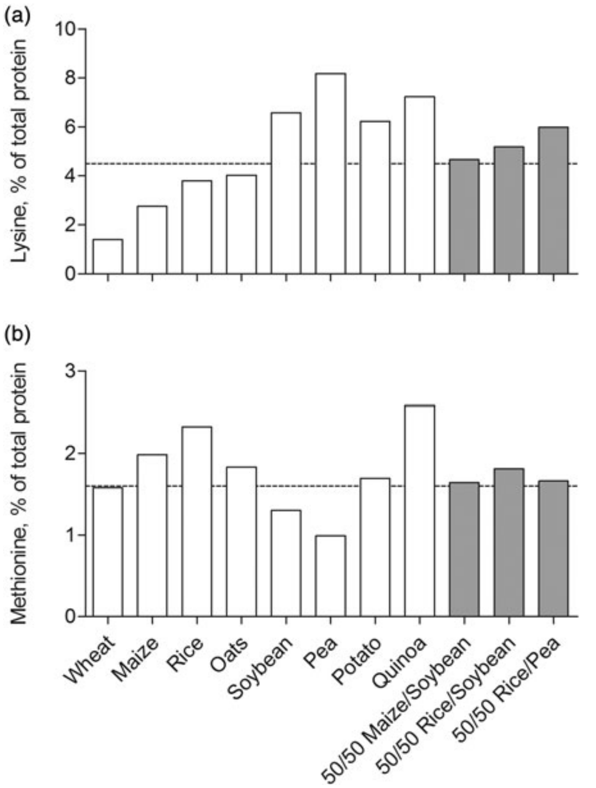

Fig. 3. Lysine (a) and methionine (b) contents (% of total protein) of various plant-based protein sources (white bars) and protein blends (grey bars). Dashed line represents the amino acid requirements for adults(52). Data are derived from FAO Nutritional Studies(88).

to providing substrate for the synthesis of new muscle protein, leucine acts as a key signal for activating the muscle protein synthetic machinery. The leucine content of animal proteins is typically 10 % or greater, whereas the leucine content of most plant proteins ranges from 6 to 8 %. An exception to this rule is maize protein, which constitutes approximately 12 % leucine. The leucine content can be modified simply by adding free leucine to the protein source. Two studies have demonstrated that adding free leucine (2·5 g) to a meal-like bolus of casein further increases the postprandial stimulation of muscle protein synthesis at rest(62,63). In addition, 2 weeks of leucine supplementation has been shown to increase both post-absorptive and postprandial muscle protein synthesis rates(64). In contrast, the addition of leucine to leucine-rich whey protein failed to further increase the postprandial muscle protein synthetic response during post-exercise recovery(65). Moreover, long-term leucine supplementation did not improve muscle mass or strength in healthy older adults who consumed an adequate amount of protein within their diet (about 1·0 g/kg per d)(66). To our knowledge, no study to date has determined whether co-ingesting leucine with a plant-based, leucine-poor, protein source potentiates the postprandial muscle protein synthetic response at rest or following exercise in older adults. In addition, no study has examined the impact of supplementing a vegetarian diet with leucine on chronic changes in muscle mass in older adults. While limited data currently exist in human subjects(67), a study in rodents demonstrated that fortification of wheat with leucine to match the leucine content of a whey protein meal resulted in a similar postprandial muscle protein synthetic response(68). Hence, it is intuitive that enriching lower leucine-containing plant-based proteins will enhance postprandial muscle protein synthesis rates in older adults.

Enhancing the muscle anabolic sensitivity to ingested protein

As an alternative approach to enhancing the anabolic capacity of plant-based proteins, increasing the sensitivity of skeletal muscle to anabolic stimuli may potentiate the postprandial muscle protein synthetic response after the ingestion of plant-based protein. The most potent approach to enhance the sensitivity of skeletal muscle is physical activity. Both resistance(60) and aerobic(69) exercise performed before protein intake have been shown to enhance the utilisation of protein-derived amino acids for *de novo* muscle protein synthesis in older adults. More recent attention has focused on the role of fish oil-derived *n*-3 PUFA for increasing the anabolic sensitivity of skeletal muscle to protein intake, with more encouraging results in older(70) compared with young adults(71). In a proof-of-concept study, 8 weeks supplementation with a daily dose of 1·9 g EPA and 1·5 g DHA was shown to potentiate muscle protein synthesis rates in response to simulated feeding (i.e. a hyperaminoacidemic-hyperinsulinemic clamp) in middle-aged and older adults(70,72). The enhanced muscle protein synthetic response to amino acid provision with *n*-3 PUFA supplementation was associated with an increased incorporation of *n*-3 PUFA into the muscle phospholipid membrane(70,72) and an increased expression and phosphorylation of anabolic signalling proteins such as mechanistic target of rapamycin complex 1, protein kinase B and focal adhesion kinase(70,72,73). Moreover, findings from a recent cell culture experiment suggests that EPA rather than DHA is the anabolically active ingredient of fish oil, both in terms of upregulating muscle protein synthesis and suppressing muscle protein breakdown(74). A next step for this research field is to investigate the role of *n*-3 PUFA supplementation in sensitising skeletal muscle to the ingestion of a plant-based protein source in older adults.

Does a plant-based diet support muscle mass maintenance?

Acute metabolic studies utilising stable isotope tracer methodology offer a powerful approach to qualitatively compare the anabolic response to the ingestion of an isolated dairy, meat, or plant-based protein source (primarily by measuring postprandial muscle protein synthesis rates)(75). However, dietary protein is generally consumed as part of a meal providing proteins from various sources. As an alternative approach to acute tracer studies, several studies have utilised dual-energy X-ray absorptiometry, computed tomography or MRI techniques to assess chronic changes (over weeks or months) in skeletal muscle mass following consumption of meat-based *v.* plant-based diets(76). For example, Campbell *et al.*(77) compared a mixed omnivorous diet (about 50 % protein from beef, pork, poultry and fish) with a lacto-ovo vegetarian diet and assessed changes in muscle mass over 12 weeks resistance training. Older men in the lacto-ovo vegetarian group did not gain fat free mass, whereas men in the omnivorous group gained 1·7 kg fat free mass on average over 12 weeks resistance training. However, the vegetarian diet provided less protein (0·8 g/kg per d) compared with the omnivorous diet (1·0 g/kg per d). When consuming 1·2 g protein/kg per d, resistance training-induced gains in muscle mass (midthigh cross-sectional area) did not differ between the vegetarian and the omnivorous diet in healthy older men(78). On a population level, cross-sectional studies showed that total protein and animal protein intake, but not plant protein intake, are positively associated with muscle mass index(79–81) and leg lean mass(82). In addition, longitudinal studies have shown that higher intakes of total protein and animal protein are associated with a reduced loss of lean mass over 3 years of follow-up(83,84) and a reduced loss of grip strength over 6 years of follow-up(85). The absence of a significant association between plant protein intake and changes in lean mass may be related to a smaller range of plant protein intakes compared with animal protein intakes and/or the inclusion of trunk lean mass measurements, which mainly includes organs rather than skeletal muscle. Interestingly, higher plant protein intake was significantly associated with a reduced loss of appendicular lean mass (including lean mass of arms and legs only) over 3 years of follow-up(84). As an alternative approach to assessing the relationship between dietary protein source and muscle mass, Mangano *et al.*(86) recently identified six food clusters each containing proteins from various sources but predominantly from (1) fast food, (2) red meat, (3) chicken, (4) fish, (5) milk or (6) legumes. Men and women in the legume group consumed high amounts of beans and peas, nuts and seeds, fruit and vegetables, and cereals, but not from red meat and relatively low amounts from dairy, chicken and fish. Participants in the legume food cluster had a lower appendicular lean mass compared with participants in the other food clusters. However, after adjusting for known confounding factors such as age, sex, BMI, physical activity level, smoking status and alcohol intake, no significant differences between food clusters were observed. Together, these data suggest that diets high in plant protein sources have the potential to support the maintenance of muscle mass with ageing provided that sufficient amounts of protein are consumed.

Conclusions

Total dietary protein intake plays a critical role in maintaining skeletal muscle mass with advancing age(87). The source of protein consumed may represent another factor that influences the preservation of muscle mass in older adults. Although a wide range of protein-rich foods are commonly consumed, scientific insight into the impact of chosen protein source on muscle protein synthesis is limited to the ingestion of milk proteins, beef and the plant-based proteins soya and wheat protein. To our knowledge, no study to date has characterised the postprandial muscle protein synthetic response to the consumption of egg, poultry, pork, fish, or plant-based proteins other than soya and wheat in older adults. Based on currently available evidence from studies in older adults, there is general consensus that on a gram-for-gram basis, the ingestion of animal-based protein sources such as dairy and meat are more potent in terms of stimulating muscle protein synthesis compared with plant-based proteins. This differential postprandial stimulation of muscle protein synthesis is likely attributed to differences in protein digestion and amino acid absorption kinetics, essential amino acid profile and leucine content between plant-based and animal-derived protein sources. However, this belief may be considered somewhat premature given that a comparison with plant-based protein sources is limited to soya and wheat proteins, while other plant-based proteins (e.g. maize or potato protein) may have a greater anabolic potential due to a favourable amino acid composition. From the standpoint of environmental sustainability and food security, plant-based protein-rich foods may be considered advantageous over animal-based protein-rich foods on a gram-for-gram basis, but not when taken into account the greater dose of protein that may be required to maximally stimulate muscle protein synthesis. Theoretical strategies to increase the anabolic potential of plant-based protein sources include fortifying plant-based proteins with leucine and consuming protein blends. Future work is warranted to develop and apply these strategies with a view to overcoming the inferior anabolic properties of plant-based proteins and help maintain muscle mass in older adults.

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