The Journal of Nutrition. First published ahead of print July 29, 2015 as doi: 10.3945/jn.114.204305.

The Journal of Nutrition
Critical Review

The Skeletal Muscle Anabolic Response to Plant- versus Animal-Based Protein Consumption[1]

Stephan van Vliet,[2,3] Nicholas A Burd,[2,3] and Luc JC van Loon[3]*

[2]Department of Kinesiology and Community Health, University of Illinois at Urbana-Champaign, Urbana, IL; and [3]Department of Human Movement Sciences, Faculty of Health, Medicine, and Life Sciences, School for Nutrition and Translational Research in Metabolism (NUTRIM), Maastricht University, Maastricht, Netherlands

Abstract

Clinical and consumer market interest is increasingly directed toward the use of plant-based proteins as dietary components aimed at preserving or increasing skeletal muscle mass. However, recent evidence suggests that the ingestion of the plant-based proteins soy and wheat results in a lower muscle protein synthetic response when compared with several animal-based proteins. The possible lower anabolic properties of plant-based protein sources may be attributed to the lower digestibility of plant-based sources, in addition to greater splanchnic extraction and subsequent urea synthesis of plant protein–derived amino acids when compared with the ingestion of animal-based proteins. The latter may be related to the relative lack of specific essential amino acids in plant- as opposed to animal-based proteins. Furthermore, most plant proteins have a relatively low leucine content, which may further reduce their anabolic properties when compared with animal proteins. However, few studies have actually assessed the postprandial muscle protein synthetic response to the ingestion of plant proteins, with soy and wheat protein being the primary sources studied. Despite the proposed lower anabolic properties of plant vs. animal proteins, various strategies may be applied to augment the anabolic properties of plant proteins. These may include the following: *1*) fortification of plant-based protein sources with the amino acids methionine, lysine, and/or leucine; *2*) selective breeding of plant sources to improve amino acid profile; *3*) consumption of greater amounts of plant-based protein sources; or *4*) combining the ingestion of multiple protein sources to provide for a more balanced amino acid profile. However, the efficacy of such dietary strategies on postprandial muscle protein synthesis remains to be studied. Future research comparing the anabolic properties of a variety of plant-based proteins should define the preferred protein sources to be used in nutritional interventions to support skeletal muscle mass gain or maintenance in both healthy and clinical populations. *J Nutr* doi: 10.3945/jn.114.204305.

Keywords: plant protein, animal protein, muscle mass, vegetarian, exercise, aging

Introduction

Skeletal muscle mass is regulated via changes in both muscle protein synthesis (MPS)[4] and muscle protein breakdown. Of the 2, the stimulation of MPS is assumed to be the primary variable responsible for regulating the maintenance or gain in skeletal muscle mass (1–4). The 2 main anabolic stimuli that augment MPS are food intake, in particular dietary protein, and physical activity (5–7). In addition to providing substrates for newly (de novo) synthesized proteins, dietary protein–derived essential amino acids (EAAs) also act as signaling molecules to induce the MPS response (8). However, the MPS response to food intake is short lived and appears to last for only 4–5 h after ingestion (9). Therefore, the consumption of protein in regular intervals throughout the course of the day is necessary to maximize daily muscle protein accretion in both postexercise (10) and resting (11) conditions. Physical activity or exercise performed before food intake has been shown to sensitize skeletal muscle tissue to the anabolic properties of protein ingestion (12). Consequently, more of the dietary protein–derived amino acids (AAs) will be used for de novo MPS when exercise is performed before protein feeding. The exercise-induced increase in anabolic sensitivity to dietary protein–derived AAs has been shown to be sustained for up to 24 h after exercise (13). Therefore, regular physical activity or exercise combined with adequate dietary protein consumption is required to preserve or increase skeletal muscle mass and strength (14).

[1] Author disclosures: S van Vliet, NA Burd, and LJC van Loon, no conflicts of interest.

[4] Abbreviations used: AA, amino acid; EAA, essential amino acid; LBM, lean body mass; MPS, muscle protein synthesis; PDCAAS, Protein Digestibility Corrected Amino Acid Score; QMP, quality maize protein; WHO/FAO/UNU, World Health Organization/Food and Agriculture Organization of the United Nations/United Nations University.

* To whom correspondence should be addressed. E-mail: l.vanloon@maastrichtuniversity.nl.

Manuscript received September 24, 2014. Initial review completed November 20, 2014. Revision accepted June 30, 2015.
doi: 10.3945/jn.114.204305.

Many health care professionals, sports nutritionists, and athletes use the knowledge gained from studies that measure the dynamics of postprandial MPS to develop nutritional strategies to facilitate maintenance or gain in skeletal muscle mass, muscle function, and/or performance in both clinical and athletic populations. So far, most studies have assessed the MPS response after the ingestion of free AAs (1–4, 15) or intact animal-based protein sources, such as milk (and its constituents whey and casein) (12, 15–25), beef (26–29), and egg (30) protein. In contrast, few studies have assessed the impact of plant-based protein ingestion on the postprandial MPS response (19, 21, 31, 32). Moreover, soy protein is the only plant-based protein that has been studied for its postprandial MPS response in humans. Studies assessing the postprandial MPS response to soy protein ingestion have shown that the ingestion of amounts ranging from 17.5 to 40 g soy protein do not increase MPS to the same extent as the ingestion of isonitrogenous amounts of whey protein (19, 21), skimmed milk (32), or beef (31), both in resting and postexercise conditions.

It is surprising that only soy protein has been applied in these types of studies, because there is considerable interest in the potential of using plant-based proteins to support muscle mass maintenance and/or growth. This belief is shown by the number of recent publications studying the impact of plant-based protein ingestion on the skeletal muscle anabolic response, provided either as an isolated protein source (16, 19, 21, 31–33) or as part of a protein blend (i.e., a combination of both plant- and animal-based proteins) (33–37).

From the standpoint of global sustainability, plant-based foods are proposed to be advantageous over animal-based foods (38–40). It has been suggested that the production of plant-based foods requires less water, land, and energy. This may ultimately pose less environmental burden and lower the financial cost of food production. These notions likely explain the increasing interest in the potential of using plant-based protein sources in clinical feeding formulas and sports nutrition supplements. In addition, there is a growing consumer market interest in plant-based foods, including plant-based meat substitutes, entreés, convenience foods, and nondairy milks (41, 42). Finally, most of the dietary protein consumed worldwide is derived from plant- as opposed to animal-based sources (~58% vs. 42%, respectively; **Table 1**) (43). Given the above, more comprehensive insight into the functional capacity of plant-based proteins to stimulate postprandial MPS is warranted. The purpose of this review is to assess the potential of plant-based protein sources to stimulate postprandial MPS and to evaluate their ability to support muscle mass maintenance/gain in both healthy and clinical populations.

Protein Quality

Dietary protein quality may refer to the ability of a protein source to support the increase in MPS after ingestion. Given the resources needed to compare the in vivo skeletal muscle anabolic properties of various protein sources, only a few research groups use contemporary stable isotope AA tracer methodology to directly assess the capacity of various dietary proteins to stimulate postprandial MPS. This likely explains the relative lack of studies comparing the postprandial MPS response to the ingestion of a wide variety of (plant)-protein sources.

Various alternative measures exist to evaluate dietary protein quality. The most widely adopted indexes are the Protein Digestibility Corrected Amino Acid Score (PDCAAS) (**Table 2**) (46) and, more recently, the Digestible Indispensable Amino Acid Score (47). However, both the PDCAAS and Digestible Indispensable Amino Acid Score do not provide insight into the true skeletal muscle "anabolic potential" of a specific dietary protein source. Instead, these ranking methods provide insight into the minimum amounts of nitrogen and AAs needed to prevent whole-body protein deficiency. For instance, soy beans have a high PDCAA score of 0.91 and are on par with beef, which scores 0.92 (Table 2). On the basis of their respective PDCAASs, one could expect soy beans to be as effective in stimulating MPS as beef. However, recent work by Phillips (31) demonstrated that beef is superior in stimulating postprandial MPS when compared with an isonitrogenous amount of a soy-based beef replacement. In agreement is the work comparing isolated soy protein with dairy proteins, which showed that, despite a similar PDCAAS (Table 2), these sources differ in their ability to stimulate MPS in both resting and postexercise conditions (19, 21, 32).

On the basis of AA tracer studies it has been established that the potential of a protein source to stimulate MPS depends on both the dietary protein digestion and AA absorption kinetics of

TABLE 1 Energy and protein consumption per capita per day[1]

	Energy intake, kJ/d	Energy intake, kcal/d	Total protein intake, g/d	Animal protein intake, % total protein intake	Plant protein intake, % total protein intake
Worldwide	10.6	2542	78	42	58
Region					
Africa	10.7	2560	67	23	77
Asia	11.3	2706	75	34	66
Americas	13.4	3205	92	56	44
Europe	14.1	3362	102	57	43
Oceania	13.9	3312	98	63	37
Economic class[2]					
Low income	10.0	2393	63	21	79
Low-middle income	10.9	2597	68	34	66
High-middle income	12.1	2907	83	46	54
High income	13.7	3296	101	58	42

[1] Daily energy intake, total daily protein intake, and the percentage of dietary protein intake provided by animal- and plant-based protein sources. The data are ranked from high to low by means of plant protein intake (%) and clustered by region or economic class. Data are from reference 43.
[2] Data on economic class are from reference 44.

TABLE 2 PDCAAS of common protein foods[1]

Source	PDCAAS
Milk	1.00
Whey	1.00
Egg	1.00
Soy protein isolate	1.00
Casein	1.00
Beef	0.92
Soy	0.91
Pea	0.67
Oat	0.57
Whole wheat	0.45

[1] The PDCAAS is a method sometimes used to assess the ability of a given protein source to support skeletal muscle anabolism. This method factors in amino acid contents and digestion kinetics. The PDCAASs of the various protein sources are ranked from high to low, where a higher score suggests a greater ability to support skeletal muscle anabolism. PDCAAS, Protein Digestibility Corrected Amino Acid Score. Data are from reference 45.

the ingested food source (17, 22–24, 48) as well as the (essential) AA composition (2, 49), with the leucine content being of particular relevance (50). In the following sections, these factors will be discussed with respect to plant-based proteins.

Protein digestion and absorption kinetics. With the use of intrinsically labeled protein sources (which have the AA tracer directly incorporated into the peptide chain of the protein), our laboratory demonstrated that the protein digestion and absorption kinetics of the ingested protein source are important for modulating postprandial MPS (17, 24). In particular, we demonstrated that enzymatic hydrolysis of intact micellar casein facilitated dietary protein digestion and AA absorption in vivo in humans (24). The ingestion of hydrolyzed vs. intact casein resulted in higher exogenous phenylalanine appearance rates, increased plasma AA availability, and a tendency to further increase postprandial MPS rates (24). Our work and that of others (51, 52) highlight that the protein digestion rate of a food source (fast vs. slow) is an important and independent predictor of postprandial MPS. In addition, this work has shown that a total of 50–70% of the dietary protein–derived AAs from milk and beef protein become available in systemic circulation when assessed over a 5- to 6-h postprandial period (17, 22, 29). After being released into the systemic circulation, these dietary protein–derived AAs have the potential to facilitate the postprandial increase in MPS.

For both methodologic and financial reasons, intrinsically labeled plant proteins are not widely available. Therefore, there are not many quantitative data describing the digestion and absorption kinetics of various plant-based proteins. However, the sparse data available suggest that the reduced ability of soy protein to support MPS as opposed to whey (21), milk (32), and beef (31) may in part be attributed to differences in digestion and absorption kinetics.

In general, it appears that plant-based protein sources may exhibit lower digestibility than animal-based proteins (53). The digestibility of the protein source has been defined as the proportion of dietary protein–derived AAs that is effectively digested and absorbed, thus becoming available in a form suitable for body protein synthesis (54). Animal-based protein sources, including dairy, eggs, and meat, are highly digestible (>90%) (53). Depending on the processing method (54) and/or the presence of various "antinutritional" factors (i.e., compounds in the food source that interfere with digestion and absorption of the available protein) (55), plant-based sources such as maize, oat, bean, pea, and potato tend to exhibit lower digestibility than do animal-based sources, with values ranging from 45% to 80% (53). However, once freed from these antinutritional factors, purified plant protein sources such as soy protein isolate, pea protein concentrate, and wheat gluten display a digestibility that is similar to that of animal-based protein sources (>90%) (53).

In addition to digestibility issues, it was reported that the dietary protein–derived AAs from the plant-based proteins soy and wheat are more readily converted to urea when compared with the ingestion of milk proteins (56–59). This would ultimately lower the potential of these plant-based protein sources to stimulate the skeletal muscle anabolic response. The exact reason(s) why the ingestion of these plant-based proteins leads to greater urea synthesis is not fully understood but may relate to the relative lack of specific EAAs. EAAs cannot be synthesized de novo in the human body and therefore have to be supplied through the diet. It is hypothesized that the ingestion of an "unbalanced" EAA profile results in an unfavorable AA mixture for gut protein synthesis, which leads to more of the free AAs to enter the portal vein to hepatic tissue (60). Ultimately, higher free AA concentrations in the liver serve as a stimulus for ureagenesis, resulting in less of the dietary-derived AAs becoming available in the systemic circulation to support the postprandial increase in MPS. This notion seems to be confirmed by animal data comparing the ingestion of crystalline AA mixtures, reflecting the AA composition of casein, egg, and wheat protein (61). It was found that the ingestion of the representative wheat mixture resulted in higher free AA concentrations in the liver and subsequent higher ureagenesis than the ingestion of the representative casein and egg mixture. Given that all mixtures were provided as free AAs, differential digestion rates could not have influenced these findings. Therefore, the observed differences in urea production are likely attributed to differences in EAA content of the different protein sources (61). In addition, recent human data by Yang et al. (21) showed that the ingestion of soy protein results in higher AA oxidation rates than does whey protein ingestion. This suggests that more of the AAs derived from soy protein were directed to urea synthesis when compared with the AAs derived from the consumption of an isonitrogenous amount of whey protein. As a consequence, less of the soy protein–derived AAs were available to stimulate MPS when compared with the whey protein–derived AAs. Because the protein digestion rates of both sources are equal (19), these findings are likely attributed to the differences in EAA content (**Table 3**, **Figure 1**). On the basis of the above, we speculate that the EAA composition of a protein source is predictive of its ability to stimulate skeletal muscle anabolism and that all EAAs should be present in "sufficient" quantities to optimally stimulate postprandial MPS.

The lysine and/or methionine contents are lower in plant-based proteins than in animal-based proteins (69). According to recommendations by the World Health Organization/Food and Agriculture Organization of the United Nations/United Nations University (WHO/FAO/UNU) (46), daily dietary requirements for lysine are ~30 mg/(kg body weight · d) per day or ~4.5% of the content of the total amount of dietary protein consumed. For methionine, daily dietary needs are estimated to be ~10 mg/(kg body weight · d) or ~1.6% of the content of the total amount of dietary protein consumed (46). Note that these percentage requirements are based on a recommended adult protein intake of 0.66 g/(kg body weight · d). Upon comparison, it becomes

TABLE 3 Amino acid concentrations of various common dietary protein sources

Source[1]	Essential amino acids, % total protein	Leucine, % total protein	Lysine, % total protein	Methionine, % total protein
Plant sources				
Spirulina[2]	41	8.5	5.2	2.0
Mycoprotein[3]	41	6.2	6.7	1.5
Lentil[2]	40	7.9	7.6	0.9
Quinoa[2]	39	7.2	6.5	2.6
Black bean[2]	39	8.4	7.3	1.6
Maize[2]	38	12.2	2.8	2.1
Soy[4]	38	8.0	6.2	1.3
Pea[5]	37	7.8	6.3	1.6
Rice[2]	37	8.2	3.8	2.2
Oat[2]	36	7.7	4.2	1.9
Hemp[6]	34	6.9	4.1	2.3
Potato[2]	33	5.2	5.7	1.7
Wheat[7]	30	6.8	2.8	1.9
Animal sources				
Whey[8]	52	13.6	10.6	2.3
Milk[8]	49	10.9	8.6	2.7
Casein[8]	48	10.2	8.1	2.7
Beef[8]	44	8.8	8.9	2.5
Egg[9]	44	8.5	7.1	3.0
Cod[10]	40	8.1	8.8	3.0
Human muscle[8]	45	9.4	8.7	2.2

[1] Human muscle is provided as a reference standard.
[2] Data from reference 62.
[3] Data from reference 63.
[4] Data from reference 19.
[5] Data from reference 64.
[6] Data from reference 65.
[7] Data from reference 66.
[8] Data from reference 67.
[9] Data from reference 30.
[10] Data from reference 68.

clear that the amounts of lysine and/or methionine present in plant-based protein sources are lower than in animal-based protein sources and human muscle protein (Table 3, **Figure 2**). The plant-based sources wheat and maize are particularly low in lysine, with 2.8% as opposed to the "required" 4.5% of lysine. Of course, the diet would only be considered low in lysine based on the assumption that all daily protein consumed is derived from wheat and/or maize. Oat, hemp, and rice are protein sources that also provide relatively low amounts of lysine (with contents of 4.2%, 4.1%, and 3.8%, respectively), especially when compared with animal-based sources and human muscle protein that have lysine contents >7%. Interestingly, the plant-based protein sources that are sufficient in lysine appear to be relatively low in methionine (Table 3, Figure 2). For instance, lentil and soy protein have particularly low methionine contents of 0.9% and 1.3% when compared with animal-based proteins and human muscle protein (>2.2%). The plant-based protein sources black bean, wheat, oat, potato, and pea can still be considered sufficient in methionine according to the WHO/FAO/UNU (46) recommendations because these protein sources have a methionine content of ~1.6%. However, the methionine content of these plant-based sources also remains well below the methionine content of animal-based protein sources and human muscle protein (>2.2%). Rice, hemp, and maize protein have methionine contents that are closer to animal-based protein sources and human muscle protein, with contents of ~2.2%. However, as mentioned previously, these specific sources are relatively low in lysine. Worth mentioning is the recent appearance of many new unconventional plant-based protein sources, including aquatic algae (e.g., spirulina), oilseeds, and single cell fungal proteins (e.g., mycoprotein). Although some of these sources may be higher in lysine and methionine than other plant protein sources, they still remain well below the amount in most animal-based protein sources (Table 3, Figure 2). For instance, spirulina has a lysine content of 5.2% and a methionine content

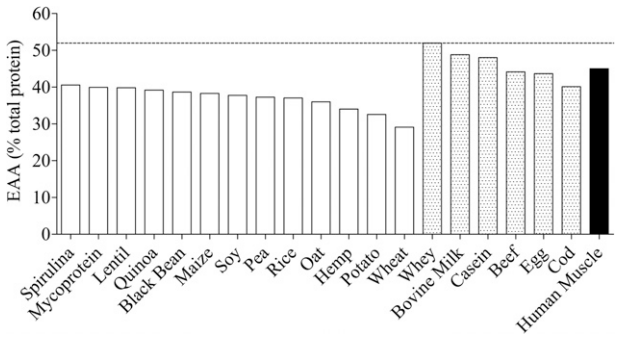

FIGURE 1 EAA concentrations of various protein sources. Differentiation is made between plant- and animal-based protein sources. Human muscle is provided as a reference standard. The dashed line represents the EAA concentration in whey protein. This line provides a comparison of the protein source most abundant in EAAs (i.e., whey) with various other protein sources. EAA, essential amino acid.

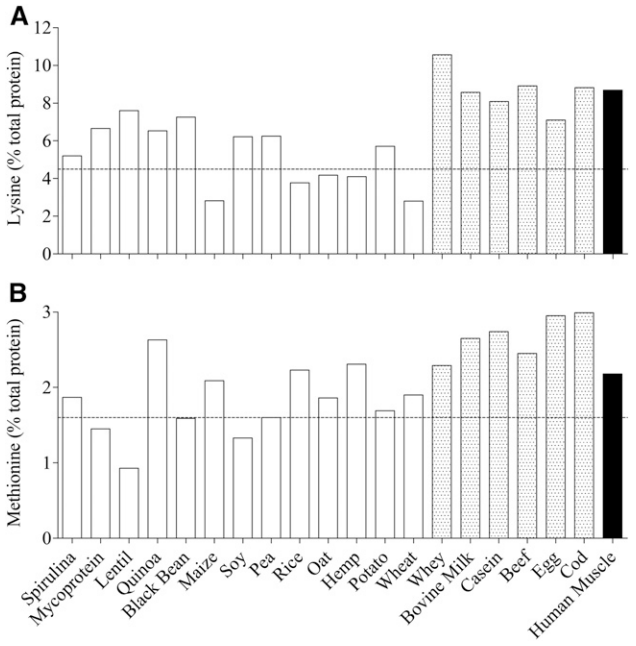

FIGURE 2 Lysine (A) and methionine (B) concentrations of various protein sources. Differentiation is made between plant- and animal-based protein sources. Human muscle is provided as a reference standard. The dashed lines represent recommendations for a minimal intake by WHO/FAO/UNU (46) guidelines. Protein sources with bars below the dashed line are considered lower than the WHO/FAO/UNU (46) requirements of the specific amino acid. WHO/FAO/UNU, World Health Organization/Food and Agriculture Organization of the United Nations/United Nations University.

of 2.0%. Although this would be in excess of the amount "required," as per WHO/FAO/UNU (46) recommendations, the lysine contents still remain well below the amounts found in animal-based proteins (which contain amounts ranging from 7% to 10%). Mycoprotein, a fungus protein often marketed as a high-quality protein source to replace animal-based protein, and in particular meat, would be considered sufficient in lysine with a level of 6.7% (63). However, like many of the other plant-based protein sources, this source is relatively low in methionine (1.5%), especially when compared with animal-based proteins (>2.2%). Of particular interest is the plant-based protein source quinoa, which can be considered a high-quality protein source on the basis of its relative high lysine (6.5%) and methionine (2.6%) contents when compared with most other plant-based protein sources. Furthermore, quinoa protein has a relatively high total EAA content (39%). It remains to be investigated whether the ingestion of quinoa or quinoa protein would translate into superior peripheral AA availability and/or greater postprandial MPS rates when compared with the ingestion of isonitrogenous amounts of other (plant) protein sources.

Leucine content. Recent studies have described leucine as the most potent AA responsible for the postprandial stimulation of MPS (50). Specifically, a large, transient increase in intracellular and/or extracellular concentrations of leucine after dietary protein ingestion is often touted to be driving this response (70–75). It is now generally believed that the leucine content of a protein source is an important and independent predictor of its capacity to stimulate postprandial MPS (50, 76, 77).

Tang et al. (19) examined the pattern of plasma AA concentrations and postprandial MPS after the ingestion of similar amounts of whey, casein, and soy protein during recovery from resistance exercise in healthy young men. It was shown that the ingestion of whey and soy protein, both acid soluble (53), resulted in a more rapid appearance of leucine in the blood than with the ingestion of casein protein. Furthermore, the highest level of leucinemia and subsequent stimulation of MPS was observed after the ingestion of whey protein (17). An interesting observation is that the consumption of soy protein led to greater MPS rates than did the consumption of the animal-derived casein protein (17). It was speculated that the slower digestion rate of casein protein and subsequent slower amino-acidemia provided a lesser stimulus for muscle anabolism than did the ingestion of soy protein. These findings further point toward the rapid, large, and transient increase in systemic leucine concentrations as an important driver of the postprandial increase in MPS.

Comparison of the different protein sources reveals that the leucine content of whey is highest, with 13.6% (Table 3, **Figure 3**). Unsurprisingly, whey protein is generally considered the superior protein source for the stimulation of postprandial MPS when compared with other rapidly digested protein sources such as soy protein isolate (19) and hydrolyzed casein (17), which have leucine contents of 8.0% and 10.2%, respectively. Animal-based protein sources generally contain more leucine than do plant-based proteins. Most plant-based sources have a leucine content of ~6–8%, whereas animal-based protein sources tend to have a leucine content in the range of 8.5–9% and >10% in the case of dairy proteins. The higher leucine content may be a key factor responsible for the proposed greater capacity of animal-based proteins to stimulate postprandial MPS rates when compared with the ingestion of various plant-based proteins (19). From this perspective, maize-derived protein represents an interesting exception because it has a relative high leucine content of 12.2%. However, comparisons of maize protein isolate to other animal- and plant-derived proteins for the stimulation of postprandial MPS remain to be conducted. On the basis of differences in leucine content between the various plant-based proteins, it could be expected that substantial differences exist in the capacity of individual plant-based protein sources to stimulate postprandial MPS rates. Research comparing the anabolic properties of the various plant- and animal-based protein sources will be of particular interest to define the preferred protein sources to be used in nutritional interventions to support skeletal muscle mass maintenance and/or to facilitate muscle hypertrophy.

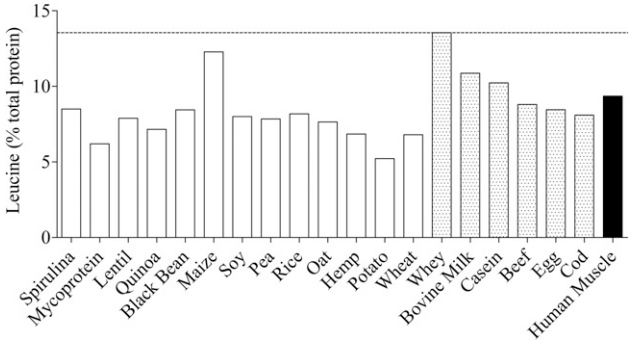

FIGURE 3 Leucine concentrations of various protein sources. Differentiation is made between plant- and animal-based protein sources. Human muscle is provided as a reference standard. The dashed line provides a comparison of the protein source most abundant in leucine (i.e., whey) with the various other protein sources.

Chronic Studies of Plant-Based Protein Intake and Muscle Mass

Acute measurements of MPS are often assumed to predict longer-term phenotypic outcomes (i.e., greater skeletal muscle maintenance/gain) to nutritional and/or exercise training interventions (78). However, acute measurements of MPS are not a quantitative estimation of muscle hypertrophy/maintenance. Instead, the acute MPS response to various nutritional and/or exercise interventions should better be viewed as an indicator of skeletal muscle reconditioning (e.g., muscle repair and remodeling), with the potential to provide insight into differences in skeletal muscle hypertrophy and/or muscle mass maintenance when performed chronically.

It has been well established that the ingestion of soy protein results in lower postprandial MPS rates than does the ingestion of beef (31), whey (19, 21), or milk (32), both at rest and during recovery from exercise. This begets the question as to whether chronic intake of plant- vs. animal-based proteins would result in divergent phenotypic outcomes (i.e., differences in muscle mass).

Hartman et al. (16) observed that the habitual consumption of 17.5 g milk protein during a 12-wk resistance exercise training intervention resulted in greater gains in lean body mass (LBM; 3.9 vs. 2.8 kg) than an isonitrogenous amount of soy protein. In agreement, Volek et al. (79) demonstrated that the ingestion of 24 g whey as opposed to soy protein resulted in greater gains in LBM (3.3 vs. 1.8 kg) after 36 wk of resistance exercise training in young men. Similarly, Campbell et al. (80) showed that the consumption of an omnivorous diet during a 12-wk resistance exercise training program induced greater gains in LBM and increases in type II fiber size than did the consumption of a predominantly lactovarian diet. Later it was demonstrated that increasing daily dietary protein intake from 0.78 g/(kg body weight · d) to 1.15 g/(kg body weight · d) eliminated the differences between the groups who consumed an omnivorous diet vs. a lactovarian diet (81). Overall, these findings imply that the ingestion of higher amounts of protein may reduce the proposed differences in the capacity of different protein sources (plant vs. animal) to modulate the gains in skeletal muscle mass during prolonged exercise interventions (82).

Indeed, it could be hypothesized that the ingestion of greater quantities of plant-based proteins may compensate for the lower EAA content, thereby improving the potential of plant-based proteins to support skeletal muscle mass gains. Joy et al. (33) recently observed that the ingestion of either 48 g rice protein or an isonitrogenous and isoenergetic amount of whey protein, immediately after resistance exercise, promoted similar increases in LBM (2.5 vs. 3.2 kg) during an 8-wk training intervention in healthy young men. Brown et al. (37) provided their subjects with either 33 g soy or whey protein and showed similar increases in muscle mass after prolonged resistance exercise training. Collectively, the studies that provided greater amounts of plant-based proteins showed minimized differences in lean mass gain with resistance exercise when compared with the ingestion of animal-based proteins. Although more evidence is required, we argue that plant-based protein supplementation can be successfully applied to support muscle mass accretion during prolonged resistance exercise, provided that greater amounts of plant-based proteins (>30 g/meal) are being consumed. Although the data above are only applicable to the specific population engaged in resistance exercise, divergent phenotypic outcomes with regard to plant- vs. animal-based protein intake have also been observed in other population groups.

A greater proportion of daily protein intake derived from animal- vs. plant-based sources is associated with better muscle maintenance in older and more clinically compromised individuals (83–86). For instance, long-term vegetarianism in older women has been reported to compromise muscle mass maintenance when compared with consumers of an omnivorous diet (18.2 vs. 22.6 kg LBM) (85). Although more long-term studies are required, it appears that prolonged (lifelong) vegetarianism can result in lower muscle mass maintenance across the life span. Aging has been associated with a progressive decline in skeletal muscle mass and appears to be driven in part by a greater anabolic resistance of skeletal muscle tissue to dietary protein ingestion (87). Given the importance of skeletal muscle mass for metabolic health and physical functioning (88), strategies to improve the sensitivity of skeletal muscle tissue to the anabolic properties of plant-based proteins could be of particular interest for aging populations. In addition, strategies to enhance the anabolic response to the ingestion of plant-based proteins may increase consumer demand, thereby supporting global sustainability, and reduce the costs associated with the production of high-quality-protein–dense foods.

Strategies to Augment the Anabolic Properties of Plant-Based Proteins

Protein quantity. As mentioned, plant-based proteins contain fewer EAAs than do animal-based proteins. This is predominantly due to the lower amounts of lysine, methionine, and/or leucine (Table 3, Figure 2). In the section above, we argued that the ingestion of greater amounts of plant-based protein per meal, consequently ingesting greater amounts of EAAs (and notably leucine), may compensate for the lower muscle anabolic properties of plant- vs. animal-based proteins. Further evidence for such a notion is found in acute work studying the postprandial MPS response to plant- vs. animal-based protein intake in an animal model. Specifically, Norton et al. (89) established that the ingestion of one-third more wheat than whey protein elicited similar postprandial MPS rates in rodents. However, recent data in elderly men suggest that the consumption of greater amounts of plant-based proteins, as a strategy to maximize postprandial MPS rates, may not per se provide a feasible solution (21). More specifically, Yang et al. (21) reported that leucine oxidation rates were elevated after the ingestion of 40 g soy protein when compared with the ingestion of a similar amount of whey protein in older individuals. These findings suggest that part of the soy protein–derived AAs are directed more toward oxidation than used for de novo MPS when compared with whey protein ingestion. Moreover, because 20 g whey and soy protein did not maximize the postprandial MPS response in these older individuals, the study provided further evidence to support the idea that aging muscle requires more leucine to maximally stimulate MPS rates (87).

Previously it was demonstrated that the postprandial MPS response in healthy young individuals is dose dependent up to ~20 g (~10 g EAAs) of high-quality, animal-based protein (25, 30, 90). In older individuals, maximal stimulation of postprandial MPS tends to be achieved with the ingestion of greater amounts of high-quality, animal-based protein (>35–40 g) (21, 23, 28). Given the lower leucine contents of plant-based protein sources, substantial amounts (>40 g) of a plant-based protein source should theoretically be ingested to maximize postprandial MPS rates in older individuals. It is evident that consuming such a large amount of protein in a single meal is far from

practical. Therefore, simply increasing the amount of dietary protein consumed per meal may not be the most feasible solution to the problem, especially in a more clinical setting in which food intake is compromised. However, we must note that research into the dose dependency of different plant-based protein sources to maximize MPS is warranted. As a reference, **Table 4** presents the amounts of various plant-based foods that should be ingested per meal to allow for the ingestion of the same amount of leucine (~3 g leucine) as is present in 23 g whey protein. Such a dose of protein/leucine was previously shown to maximize postprandial MPS in young individuals both in rested and postexercise conditions (25).

Leucine fortification. The leucine content of a meal appears to be of fundamental importance to postprandial stimulation of MPS, especially in older populations (50) and in more clinically compromised patient groups (91). Previously, Rieu et al. (72) found that approximately doubling the leucine content of mixed meals increased MPS rates in older individuals. Similar observations were reported by Katsanos et al. (75), who showed that increasing the leucine content of an AA mixture reversed the blunted postprandial MPS response in older subjects. We recently investigated the impact of ingesting intrinsically L-[1-^{13}C]phenylalanine–labeled micellar casein with or without the addition of free leucine on postprandial MPS in elderly individuals. The coingestion of free leucine (2.5 g) with 20 g of casein led to greater postprandial muscle protein accretion than did the ingestion of casein only (92). Moreover, these stimulating effects of leucine are likely maintained with chronic administration of a higher leucine diet. Casperson et al. (93) observed increases in both postabsorptive and postprandial MPS rates after 2 wk of leucine supplementation (4 g/meal for 3 meals daily). However, the clinical relevance of leucine supplementation as a dietary strategy to gain muscle mass remains questionable. We failed to demonstrate measurable increases in skeletal muscle mass or strength after 3 and 6 mo of leucine supplementation of 7.5 g/d in both healthy (94) and type 2 diabetic (95) elderly men. The lack of impact of prolonged leucine supplementation on skeletal muscle mass or strength may be attributed to the fact that the healthy and type 2 diabetic elderly men were habitually active and were already consuming ample amounts of dietary protein, namely >1 g/(kg body weight · d).

A possible issue with crystalline leucine supplementation to the diet may be the plasma- and tissue-depleting effects on the other BCAAs (valine and isoleucine). For instance, we observed a rapid decline in fasting plasma valine (−23% ± 2%) and isoleucine (−16% ± 2%) concentrations within the first 4 wk of intervention (95). Thereafter, plasma valine and isoleucine concentrations remained stable. Whether this decline in fasting plasma valine and isoleucine concentration is of physiologic relevance remains debatable, because the basal concentrations did not decline further and always remained within a normal physiologic range. Moreover, many studies showed an improvement in postprandial MPS with several grams of free leucine supplementation (72, 75, 92, 93). These data suggest that the addition of a few grams of crystalline AAs to a dietary protein source is unlikely to negatively affect protein metabolism and may in fact improve the skeletal muscle anabolic response in settings in which dietary protein intake is (substantially) lowered.

Work assessing the acute or long-term muscle anabolic response in humans to the ingestion of plant-based protein sources fortified with free leucine is currently limited. However,

TABLE 4 Amount of dietary protein to, theoretically, maximize postprandial MPS[1]

Source	Leucine, % total protein	Representative amount of protein to be ingested per meal for ~3 g leucine, g	Representative amount of the food source to be ingested per meal, g
Plant sources			
Maize	12.3	25	264
Spirulina	8.5	36	63
Black bean	8.4	36	167
Rice	8.2	37	500
Soy	8.0	38	104
Lentil	7.9	39	150
Pea	7.8	39	180
Oat	7.7	35	236
Quinoa	7.2	43	302
Hemp	6.9	45	121
Wheat	6.8	45	299
Mycoprotein	6.2	49	447
Potato	5.2	58	2891
Animal sources			
Whey	13.6	23	27
Milk	10.9	28	876
Casein	10.2	30	35
Beef	8.8	35	164
Egg	8.5	36	5[2]
Cod	8.1	38	211

[1] Amount of protein source to be ingested to maximize postexercise MPS rates in response to feeding in young subjects. Data are ranked from high to low by leucine content. A higher leucine content suggests that a lower amount of dietary protein from a given source is needed to maximize postprandial MPS rates. The third column (amount of protein to be ingested per meal) represents a theoretical value using whey protein as a standard of reference. The amounts of protein calculated represent the amount needed to match the leucine content found in 23 g whey protein (~3 g). The representative amounts for whey and casein assume isolated protein sources, whereas all other protein sources are expressed as representative amounts of the intact food source. MPS, muscle protein synthesis.
[2] Number of eggs.

there is evidence to suggest that the addition of free leucine to plant-based proteins may be a viable option to improve its anabolic properties. Engelen et al. (96) reported that supplementing a soy-based protein meal with free BCAAs (leucine, isoleucine, and valine) reduced splanchnic extraction and urea synthesis, consequently shifting dietary protein–derived AAs toward peripheral (i.e., skeletal muscle) tissue. In addition, animal work by Norton et al. (66) demonstrated that adding free leucine to wheat protein, to match the leucine content present in an isonitrogenous amount of whey protein, resulted in similar postprandial MPS rates. Overall, the addition of a several grams of free leucine to plant-based protein formulas and foods may provide an effective strategy to enhance the anabolic properties of plant-based protein sources.

Lysine and methionine fortification. As described previously, plant-based protein sources are generally lower in lysine and/or methionine than are animal-based protein sources (Table 3, Figure 2). This may impair the postprandial MPS response after the ingestion of plant-based protein sources when compared with animal-based proteins (56–59). Thus, similar to leucine fortification, it may be hypothesized that adding lysine and/or methionine to plant-based proteins is a useful strategy to improve their anabolic properties. However, work studying the postprandial MPS response to the addition of crystalline lysine and/or methionine to a plant-based protein source is currently unavailable. Nonetheless, some evidence exists that lysine fortification may be a worthwhile strategy to investigate. Such evidence can be found in studies documenting differential growth rates among young children that consume diets predominantly rich in grains (>50%), either with or without lysine fortification (97–99).

The first large lysine fortification trials were performed in the late 1960s and early 1970s (100–102). However, these studies failed to reveal a beneficial effect of lysine fortification of rice, maize, or wheat on the growth rates of children. A decade later, thorough review of these studies revealed flaws in terms of design and conduct (103). Not only did the control and intervention groups differ vastly in terms of health and socioeconomic status, in addition to geographical location, the researchers also did not control for total protein intake or energy status and/or monitor food consumption. These factors may have confounded the findings, thereby making it difficult to draw firm conclusions with regard to the impact of lysine fortification as a nutritional strategy to augment growth rates (103).

In later, well-controlled experiments, beneficial effects of lysine fortification have been observed. For instance, Zhao et al. (97) demonstrated that the fortification of wheat with free lysine, thereby increasing the lysine content of wheat protein from 2.5% to 5.5%, resulted in greater gains in height and weight of infants over a 3-mo period when compared with the ingestion of an isonitrogenous and isoenergetic nonfortified control. Moreover, the authors observed a trend for decreased triceps skinfold thickness, suggesting that the weight gain was attributed to increases in LBM rather than fat mass (97). Of note, daily protein intake was ~50 g in both groups, with as much as ~34 g derived from wheat protein. Similar findings were made in other work that examined the growth rates of infants with the consumption of lysine-fortified wheat (98) and lysine-fortified maize (99). Taken together, these findings provide additional support for the notion that the fortification of plant-based foods with crystalline AAs may improve their anabolic properties. However, future work comparing the fortification of plant-based protein sources with free AAs vs. the respective unfortified source with regard to postprandial MPS response should be conducted to confirm the validity of such a strategy to improve the skeletal muscle anabolic response.

Selective breeding of plants to improve AA composition. Selective breeding (or genetic manipulation) may also serve as a useful strategy to improve the EAA composition and digestibility of plant-based proteins and, as such, improve their anabolic properties. An example of selective breeding as a method of improving protein quality of plant-based protein is "quality maize protein" (QMP). QMP has been produced by selective breeding of maize with a single gene mutation that results in increased lysine (and tryptophan) content (104). QMP has a lysine content of ~4.2% (105), which is near the "required" 4.5% as per WHO/FAO/UNU recommendations (46). Although QMP has nearly double the lysine content than generic maize (containing ~2.3% lysine), the lysine content still remains well below the amounts reported for animal-based proteins (>7%). So far, the postprandial MPS response to the ingestion of QMP has not been assessed. Nonetheless, QMP consumption has been shown to augment growth rates of infants in underdeveloped countries, where maize comprises a large majority of daily protein intake (106). Thus, the application of selective breeding or a genetic enhancement strategy to increase the EAA content may represent an effective strategy to improve the skeletal muscle anabolic response to plant protein ingestion and could be of particular relevance in populations who consume diets rich in plant-based foods (i.e., grains) (Table 1).

Protein blends. Several recent articles have investigated the anabolic properties of ingesting a combination of plant and dairy proteins in a single meal (i.e., protein blends) (34–36, 107). For example, Reidy et al. (107) recently reported that protein blends may be useful to facilitate the postprandial MPS response during recovery from a single bout of resistance exercise in healthy young men. The authors reported no measurable differences in the MPS response during the 4-h postexercise recovery period with the ingestion of either 17.7 g whey protein or 19.3 g of a protein blend (containing a mixture of 25% whey, 25% soy, and 50% casein protein). These results are not surprising given that nearly equal amounts of EAAs (8.9 ± 0.4 vs. 8.7 ± 0.5 g) and leucine (1.9 ± 0.1 vs. 1.8 ± 0.1 g) were provided. It is evident that plant-based proteins will contribute to the postprandial increase in MPS and that they can be applied effectively in protein blends designed to support muscle mass gains. This is of particular relevance in postexercise conditions in which the muscle is even more sensitive to the anabolic properties of AAs (13, 17). However, animal-based proteins, and dairy proteins in particular, contain higher amounts of leucine than do similar amounts of plant-based proteins (Table 3, Figure 3). Consequently, protein blends containing substantial amounts of plant-based proteins (>50%) may contain less leucine than an isonitrogenous amount of an animal-based protein source. A reduction in the amount of leucine consumed may have relatively little impact on the postprandial increase in MPS rates during postexercise recovery in healthy young individuals (108). However, consuming a protein source or protein blend with a low leucine content will likely be of greater impact on stimulating postprandial muscle protein accretion in older and more clinically compromised populations. As mentioned, these individuals require greater amounts of leucine (>2.5–3g) to maximize postprandial MPS rates (50, 91).

Although the consumption of a mixture of plant- and animal-based protein sources is generic to most individuals, vegans consume a strictly plant-based diet. In the case of plant-based

protein blends it may be that the consumption of a well-balanced combination of multiple plant-based protein sources, to allow for ingestion of a "complete" EAA profile, may improve the postprandial MPS response when compared with the ingestion of a single plant-based protein source. In general, plant-based proteins are only low in 1 or 2 EEAs (Table 3, Figure 2). Thus, combining plant proteins that are lower in lysine yet higher in methionine (e.g., wheat, rice, hemp, and maize) with plant proteins that are higher in lysine yet lower in methionine (including black bean, oat, soy, lentil, potato, and pea) may augment the anabolic properties of plant-based protein intake. Research is warranted to assess whether the ingestion of a plant-based protein blend, designed to provide for a more balanced EAA profile, increases postprandial MPS rates when compared with the ingestion of a single plant-based protein source.

Conclusions

Only a few studies have compared the postprandial MPS response to the ingestion of plant- vs. animal-based proteins. To date, the only plant-based protein source that has been extensively studied in an in vivo human model is soy protein. In this work, the consumption of soy protein was demonstrated to result in lower MPS rates than the ingestion of whey, milk, or beef protein. In addition, the acute skeletal muscle anabolic response was reduced with wheat protein intake when compared with the consumption of egg or whey protein in a rodent model. The proposed lower muscle anabolic properties of plant- as opposed to animal-based protein sources may be attributed to differences in protein digestion and AA absorption kinetics, and/or AA composition. Various strategies that may improve the MPS response after the ingestion of plant-based proteins include the fortification of plant proteins with free AAs, the blending of various plant protein sources to create a more complete AA profile, selective breeding of plants to improve AA composition, and/or the consumption of greater amounts of plant proteins. Research is required to compare the anabolic properties of the many different plant-based protein sources and to assess the potential of the various strategies that may augment the postprandial MPS response to the ingestion of plant-based proteins.

Acknowledgments

SvV, NAB, and LJCvL wrote the manuscript; and SvV and LJCvL had primary responsibility for the final content. All authors read and approved the final manuscript.

References

1. Biolo G, Declan Fleming RY, Wolfe RR. Physiologic hyperinsulinemia stimulates protein synthesis and enhances transport of selected amino acids in human skeletal muscle. J Clin Invest 1995;95:811–9.
2. Volpi E, Kobayashi H, Sheffield-Moore M, Mittendorfer B, Wolfe RR. Essential amino acids are primarily responsible for the amino acid stimulation of muscle protein anabolism in healthy elderly adults. Am J Clin Nutr 2003;78:250–8.
3. Volpi E, Ferrando AA, Yeckel CW, Tipton KD, Wolfe RR. Exogenous amino acids stimulate net muscle protein synthesis in the elderly. J Clin Invest 1998;101:2000–7.
4. Wolfe RR. Regulation of muscle protein by amino acids. J Nutr 2002;132: Suppl:3219S–24S.
5. Koopman R, van Loon LJC. Aging, exercise, and muscle protein metabolism. J Appl Physiol 2009;106:2040–8.
6. Paddon-Jones D, Short KR, Campbell WW, Volpi E, Wolfe RR. Role of dietary protein in the sarcopenia of aging. Am J Clin Nutr 2008;87: Suppl:1562S–6S.
7. Phillips SM. The science of muscle hypertrophy: making dietary protein count. Proc Nutr Soc 2011;70:100–3.
8. Atherton PJ, Smith K, Etheridge T, Rankin D, Rennie MJ. Distinct anabolic signalling responses to amino acids in C2C12 skeletal muscle cells. Amino Acids 2010;38:1533–9.
9. Moore DR, Tang JE, Burd NA, Rerecich T, Tarnopolsky MA, Phillips SM. Differential stimulation of myofibrillar and sarcoplasmic protein synthesis with protein ingestion at rest and after resistance exercise. J Physiol 2009;587:897–904.
10. Areta JL, Burke LM, Ross ML, Camera DM, West DW, Broad EM, Jeacocke NA, Moore DR, Stellingwerff T, Phillips SM, et al. Timing and distribution of protein ingestion during prolonged recovery from resistance exercise alters myofibrillar protein synthesis. J Physiol 2013;591:2319–31.
11. Mamerow MM, Mettler JA, English KL, Casperson SL, Arentson-Lantz E, Sheffield-Moore M, Layman DK, Paddon-Jones D. Dietary protein distribution positively influences 24-h muscle protein synthesis in healthy adults. J Nutr 2014;144:876–80.
12. Pennings B, Koopman R, Beelen M, Senden JM, Saris WH, van Loon LJ. Exercising before protein intake allows for greater use of dietary protein–derived amino acids for de novo muscle protein synthesis in both young and elderly men. Am J Clin Nutr 2011;93:322–31.
13. Burd NA, West DWD, Moore DR, Atherton PJ, Staples AW, Prior T, Tang JE, Rennie MJ, Baker SK, Phillips SM. Enhanced amino acid sensitivity of myofibrillar protein synthesis persists for up to 24 h after resistance exercise in young men. J Nutr 2011;141:568–73.
14. Cermak NM, Res PT, de Groot LC, Saris WH, van Loon LJ. Protein supplementation augments the adaptive response of skeletal muscle to resistance-type exercise training: a meta-analysis. Am J Clin Nutr 2012;96:1454–64.
15. Paddon-Jones D, Sheffield-Moore M, Katsanos CS, Zhang X-J, Wolfe RR. Differential stimulation of muscle protein synthesis in elderly humans following isocaloric ingestion of amino acids or whey protein. Exp Gerontol 2006;41:215–9.
16. Hartman JW, Tang JE, Wilkinson SB, Tarnopolsky MA, Lawrence RL, Fullerton AV, Phillips SM. Consumption of fat-free fluid milk after resistance exercise promotes greater lean mass accretion than does consumption of soy or carbohydrate in young, novice, male weightlifters. Am J Clin Nutr 2007;86:373–81.
17. Pennings B, Boirie Y, Senden JM, Gijsen AP, Kuipers H, van Loon LJ. Whey protein stimulates postprandial muscle protein accretion more effectively than do casein and casein hydrolysate in older men. Am J Clin Nutr 2011;93:997–1005.
18. Tang JE, Manolakos JJ, Kujbida GW, Lysecki PJ, Moore DR, Phillips SM. Minimal whey protein with carbohydrate stimulates muscle protein synthesis following resistance exercise in trained young men. Appl Physiol Nutr Metab 2007;32:1132–8.
19. Tang JE, Moore DR, Kujbida GW, Tarnopolsky MA, Phillips SM. Ingestion of whey hydrolysate, casein, or soy protein isolate: effects on mixed muscle protein synthesis at rest and following resistance exercise in young men. J Appl Physiol 2009;107:987–92.
20. Tipton KD, Elliott TA, Cree MG, Aarsland AA, Sanford AP, Wolfe RR. Stimulation of net muscle protein synthesis by whey protein ingestion before and after exercise. Am J Physiol Endocrinol Metab 2007;292:E71–6.
21. Yang Y, Churchward-Venne TA, Burd NA, Breen L, Tarnopolsky MA, Phillips SM. Myofibrillar protein synthesis following ingestion of soy protein isolate at rest and after resistance exercise in elderly men. Nutr Metab (Lond) 2012;9:57.
22. Koopman R, Walrand S, Beelen M, Gijsen AP, Kies AK, Boirie Y, Saris WHM, van Loon LJC. Dietary protein digestion and absorption rates and the subsequent postprandial muscle protein synthetic response do not differ between young and elderly men. J Nutr 2009;139:1707–13.
23. Burd NA, Pennings B, Groen BB, Gijsen AP, Senden JM, van Loon LJ. The single biopsy approach is reliable for the measurement of muscle protein synthesis rates in vivo in older men. J Appl Physiol 2012;113:896–902.
24. Koopman R, Crombach N, Gijsen AP, Walrand S, Fauquant J, Kies AK, Lemosquet S, Saris WH, Boirie Y, van Loon LJ. Ingestion of a protein hydrolysate is accompanied by an accelerated in vivo digestion and absorption rate when compared with its intact protein. Am J Clin Nutr 2009;90:106–15.
25. Witard OC, Jackman SR, Breen L, Smith K, Selby A, Tipton KD. Myofibrillar muscle protein synthesis rates subsequent to a meal in response to increasing doses of whey protein at rest and after resistance exercise. Am J Clin Nutr 2014;99:86–95.

26. Symons TB, Sheffield-Moore M, Wolfe RR, Paddon-Jones D. A moderate serving of high-quality protein maximally stimulates skeletal muscle protein synthesis in young and elderly subjects. J Am Diet Assoc 2009;109:1582–6.
27. Symons TB, Sheffield-Moore M, Mamerow M, Wolfe R, Paddon-Jones D. The anabolic response to resistance exercise and a protein-rich meal is not diminished by age. J Nutr Health Aging 2011;15:376–81.
28. Robinson MJ, Burd NA, Breen L, Rerecich T, Yang Y, Hector AJ, Baker SK, Phillips SM. Dose-dependent responses of myofibrillar protein synthesis with beef ingestion are enhanced with resistance exercise in middle-aged men. Appl Physiol Nutr Metab 2013;38:120–5.
29. Pennings B, Groen BB, van Dijk J-W, de Lange A, Kiskini A, Kuklinski M, Senden JM, van Loon LJ. Minced beef is more rapidly digested and absorbed than beef steak, resulting in greater postprandial protein retention in older men. Am J Clin Nutr 2013;98:121–8.
30. Moore DR, Robinson M, Fry J, Tang J, Glover E, Wilkinson S, Prior T, Tarnopolsky M, Phillips S. Ingested protein dose response of muscle and albumin protein synthesis after resistance exercise in young men. Am J Clin Nutr 2009;89:161–8.
31. Phillips SM. Nutrient-rich meat proteins in offsetting age-related muscle loss. Meat Sci 2012;92:174–8.
32. Wilkinson SB, Tarnopolsky MA, MacDonald MJ, MacDonald JR, Armstrong D, Phillips SM. Consumption of fluid skim milk promotes greater muscle protein accretion after resistance exercise than does consumption of an isonitrogenous and isoenergetic soy-protein beverage. Am J Clin Nutr 2007;85:1031–40.
33. Joy JM, Lowery RP, Wilson JM, Purpura M, De Souza EO, Wilson SM, Kalman DS, Dudeck JE, Jager R. The effects of 8 weeks of whey or rice protein supplementation on body composition and exercise performance. Nutr J 2013;12:86.
34. Olza J, Mesa MD, Poyatos RM, Aguilera CM, Moreno-Torres R, Perez M, Perez de la Cruz A, Gil A. A specific protein-enriched enteral formula decreases cortisolemia and improves plasma albumin and amino acid concentrations in elderly patients. Nutr Metab (Lond) 2010;7:58.
35. Meneses JO, Foulquie JP, Valero GU, de Victoria EM, Hernandez AG. Biological evaluation of a protein mixture intended for enteral nutrition. Nutr Hosp 2008;23:206–11.
36. Kalman D, Feldman S, Martinez M, Krieger DR, Tallon MJ. Effect of protein source and resistance training on body composition and sex hormones. J Int Soc Sports Nutr 2007;4:4.
37. Brown EC, DiSilvestro R, Babaknia A, Devor S. Soy versus whey protein bars: effects on exercise training impact on lean body mass and antioxidant status. Nutr J 2004;3:22.
38. Pimentel D, Pimentel M. Sustainability of meat-based and plant-based diets and the environment. Am J Clin Nutr 2003;78: Suppl:660S–3S.
39. Baroni L, Cenci L, Tettamanti M, Berati M. Evaluating the environmental impact of various dietary patterns combined with different food production systems. Eur J Clin Nutr 2007;61:279–86.
40. Marlow HJ, Hayes WK, Soret S, Carter RL, Schwab ER, Sabaté J. Diet and the environment: does what you eat matter? Am J Clin Nutr 2009;89: Suppl:1699S–703S.
41. Mintel International GroupVegetarian foods (processed)—US—June. London; 2007 [cited 2014 Sep 24]. Available from: www.mintel.com.
42. Mintel International GroupMeat alternatives—US. June. London; 2013 [cited 2014 Sep 24]. Available from: www.mintel.com.
43. FAO UN Statistics Division (FAOSTAT). Food balance sheets. Rome (Italy): FAOSTAT; 2009.
44. World BankList of economies. Washington (DC); 2009 [cited 2014 Sep 24]. Available from: www.worldbank.com.
45. Schaafsma G. The protein digestibility-corrected amino acid score. J Nutr 2000;130: Suppl:1865S–7S.
46. WHO/FAO/UNU. Protein and amino acid requirements in human nutrition: report of a joint WHO/FAO/UNU expert consultation. World Health Organ Tech Rep Ser 2007;935:1–265.
47. FAO. Dietary protein evaluation in human nutrition: report of an FAO Expert Consultation. Rome (Italy): FAO; 2013.
48. Pennings B, Pellikaan WF, Senden JMG, van Vuuren AM, Sikkema J, van Loon LJC. The production of intrinsically labeled milk and meat protein is feasible and provides functional tools for human nutrition research. J Dairy Sci 2011;94:4366–73.
49. Tipton KD, Gurkin BE, Matin S, Wolfe RR. Nonessential amino acids are not necessary to stimulate net muscle protein synthesis in healthy volunteers. J Nutr Biochem 1999;10:89–95.
50. van Loon LJC. Leucine as a pharmaconutrient in health and disease. Curr Opin Clin Nutr Metab Care 2012;15:71–7.
51. Boirie Y, Dangin M, Gachon P, Vasson MP, Maubois JL, Beaufrere B. Slow and fast dietary proteins differently modulate postprandial protein accretion. Proc Natl Acad Sci USA 1997;94:14930–5.
52. Dangin M, Boirie Y, Garcia-Rodenas C, Gachon P, Fauquant J, Callier P, Ballevre O, Beaufrere B. The digestion rate of protein is an independent regulating factor of postprandial protein retention. Am J Physiol Endocrinol Metab 2001;280:E340–8.
53. FAO. Report of a sub-committee of the 2011 FAO Consultation on "Protein Quality Evaluation in Human Nutrition": the assessment of amino acid digestibility in foods for humans and including a collation of published ileal amino acid digestibility data for human foods. Rome (Italy): FAO; 2012.
54. Rutherfurd SM, Moughan PJ. Available versus digestible dietary amino acids. Br J Nutr 2012;108: Suppl 2:S298–305.
55. Sarwar Gilani G, Wu Xiao C, Cockell KA. Impact of antinutritional factors in food proteins on the digestibility of protein and the bioavailability of amino acids and on protein quality. Br J Nutr 2012;108: Suppl 2:S315–32.
56. Fouillet H, Mariotti F, Gaudichon C, Bos C, Tomé D. Peripheral and splanchnic metabolism of dietary nitrogen are differently affected by the protein source in humans as assessed by compartmental modeling. J Nutr 2002;132:125–33.
57. Bos C, Metges CC, Gaudichon C, Petzke KJ, Pueyo ME, Morens C, Everwand J, Benamouzig R, Tomé D. Postprandial kinetics of dietary amino acids are the main determinant of their metabolism after soy or milk protein ingestion in humans. J Nutr 2003;133:1308–15.
58. Bos C, Juillet B, Fouillet H, Turlan L, Daré S, Luengo C, N'tounda R, Benamouzig R, Gausserès N, Tomé D, et al. Postprandial metabolic utilization of wheat protein in humans. Am J Clin Nutr 2005;81:87–94.
59. Fouillet H, Juillet B, Gaudichon C, Mariotti F, Tomé D, Bos C. Absorption kinetics are a key factor regulating postprandial protein metabolism in response to qualitative and quantitative variations in protein intake. Am J Physiol Regul Integr Comp Physiol 2009;297: R1691–705.
60. Soeters PB, de Jong CH, Deutz NE. The protein sparing function of the gut and the quality of food protein. Clin Nutr 2001;20:97–9.
61. Tujioka K, Ohsumi M, Hayase K, Yokogoshi H. Effect of the quality of dietary amino acids composition on the urea synthesis in rats. J Nutr Sci Vitaminol (Tokyo) 2011;57:48–55.
62. USDA, Agricultural Research Service. National Nutrient Database for Standard Reference, release 26. Washington (DC): USDA; 2013 [cited 2014 Sep 24]. Available from: http://www.ars.usda.gov/ba/bhnrc/ndl.
63. Ahangi Z, Shojaosadati SA, Nikoopour H. Study of mycoprotein production using Fusarium oxysporum PTCC 5115 and reduction of its RNA content. Pakistan J Nutr 2008;7:240–3 [cited 2014 Sep 24]. Available from: http://www.pjbs.org/pjnonline/.
64. Khattab RY, Arntfield SD, Nyachoti CM. Nutritional quality of legume seeds as affected by some physical treatments, Part 1: protein quality evaluation. LWT Food Sci Technol 2009;42:1107–12 [cited 2014 Sep 24]. Available from: http://www.journals.elsevier.com/lwt-food-science-and-technology.
65. Callaway JC. Hempseed as a nutritional resource: an overview. Euphytica 2004;140:65–72.
66. Norton LE, Wilson GJ, Layman DK, Moulton CJ, Garlick PJ. Leucine content of dietary proteins is a determinant of postprandial skeletal muscle protein synthesis in adult rats. Nutr Metab (Lond) 2012;9:67.
67. Burd NA, Hamer HM, Pennings B, Pellikaan WF, Senden JMG, Gijsen AP, van Loon LJC. Substantial differences between organ and muscle specific tracer incorporation rates in a lactating dairy cow. PLoS One 2013;8:e68109.
68. Vikøren LA, Nygard OK, Lied E, Rostrup E, Gudbrandsen OA. A randomised study on the effects of fish protein supplement on glucose tolerance, lipids and body composition in overweight adults. Br J Nutr 2013;109:648–57.
69. Young VR, Pellett PL. Plant proteins in relation to human protein and amino acid nutrition. Am J Clin Nutr 1994;59: Suppl:1203S–12S.

70. Koopman R, Wagenmakers AJ, Manders RJ, Zorenc AH, Senden JM, Gorselink M, Keizer HA, van Loon LJC. Combined ingestion of protein and free leucine with carbohydrate increases postexercise muscle protein synthesis in vivo in male subjects. Am J Physiol Endocrinol Metab 2005;288:E645–53.
71. Dardevet D, Sornet C, Bayle G, Prugnaud J, Pouyet C, Grizard J. Postprandial stimulation of muscle protein synthesis in old rats can be restored by a leucine-supplemented meal. J Nutr 2002;132:95–100.
72. Rieu I, Balage M, Sornet C, Giraudet C, Pujos E, Grizard J, Mosoni L, Dardevet D. Leucine supplementation improves muscle protein synthesis in elderly men independently of hyperaminoacidaemia. J Physiol 2006;575:305–15.
73. Frexes-Steed M, Lacy DB, Collins J, Abumrad NN. Role of leucine and other amino acids in regulating protein metabolism in vivo. Am J Physiol 1992;262:E925–35.
74. Anthony JC, Anthony TG, Layman DK. Leucine supplementation enhances skeletal muscle recovery in rats following exercise. J Nutr 1999;129:1102–6.
75. Katsanos CS, Kobayashi H, Sheffield-Moore M, Aarsland A, Wolfe RR. A high proportion of leucine is required for optimal stimulation of the rate of muscle protein synthesis by essential amino acids in the elderly. Am J Physiol Endocrinol Metab 2006;291:E381–7.
76. Garlick PJ. The role of leucine in the regulation of protein metabolism. J Nutr 2005;135: Suppl:1553S–6S.
77. Millward DJ, Layman DK, Tomé D, Schaafsma G. Protein quality assessment: impact of expanding understanding of protein and amino acid needs for optimal health. Am J Clin Nutr 2008;87: Suppl:1576S–81S.
78. Mitchell CJ, Churchward-Venne TA, Cameron-Smith D, Phillips SM. What is the relationship between the acute muscle protein synthetic response and changes in muscle mass? J Appl Physiol (1985) 2015;118: 495–7.
79. Volek JS, Volk BM, Gomez AL, Kunces LJ, Kupchak BR, Freidenreich DJ, Aristizabal JC, Saenz C, Dunn-Lewis C, Ballard KD, et al. Whey protein supplementation during resistance training augments lean body mass. J Am Coll Nutr 2013;32:122–35.
80. Campbell WW, Barton ML, Jr., Cyr-Campbell D, Davey SL, Beard JL, Parise G, Evans WJ. Effects of an omnivorous diet compared with a lactoovovegetarian diet on resistance-training-induced changes in body composition and skeletal muscle in older men. Am J Clin Nutr 1999;70:1032–9.
81. Haub MD, Wells AM, Tarnopolsky MA, Campbell WW. Effect of protein source on resistive-training-induced changes in body composition and muscle size in older men. Am J Clin Nutr 2002;76:511–7.
82. Campbell WW, Leidy HJ. Dietary protein and resistance training effects on muscle and body composition in older persons. J Am Coll Nutr 2007;26:696S–703S.
83. Lord C, Chaput JP, Aubertin-Leheudre M, Labonte M, Dionne IJ. Dietary animal protein intake: association with muscle mass index in older women. J Nutr Health Aging 2007;11:383–7.
84. Maltais ML, Leblanc S, Archambault-Therrien C, Jean B, Bobeuf F, Dionne IJ. Various sources of animal protein intake and their association with muscle mass index and insulin resistance in overweight postmenopausal women. Int J Nutr Metabol 2013;5:17–21.
85. Aubertin-Leheudre M, Adlercreutz H. Relationship between animal protein intake and muscle mass index in healthy women. Br J Nutr 2009;102:1803–10.
86. Houston DK, Nicklas BJ, Ding J, Harris TB, Tylavsky FA, Newman AB, Lee JS, Sahyoun NR, Visser M, Kritchevsky SB. Dietary protein intake is associated with lean mass change in older, community-dwelling adults: the Health, Aging, and Body Composition (Health ABC) Study. Am J Clin Nutr 2008;87:150–5.
87. Burd NA, Gorissen SH, van Loon LJ. Anabolic resistance of muscle protein synthesis with aging. Exerc Sport Sci Rev 2013; 41:169–73.
88. Wolfe RR. The underappreciated role of muscle in health and disease. Am J Clin Nutr 2006;84:475–82.
89. Norton LE, Layman DK, Bunpo P, Anthony TG, Brana DV, Garlick PJ. The leucine content of a complete meal directs peak activation but not duration of skeletal muscle protein synthesis and mammalian target of rapamycin signaling in rats. J Nutr 2009; 139:1103–9.
90. Cuthbertson D, Smith K, Babraj J, Leese G, Waddell T, Atherton P, Wackerhage H, Taylor PM, Rennie MJ. Anabolic signaling deficits underlie amino acid resistance of wasting, aging muscle. FASEB J 2005;19:422–4.
91. Jonker R, Engelen MP, Deutz NE. Role of specific dietary amino acids in clinical conditions. Br J Nutr 2012;108: Suppl 2:S139–48.
92. Wall BT, Hamer HM, de Lange A, Kiskini A, Groen BBL, Senden JMG, Gijsen AP, Verdijk LB, van Loon LJC. Leucine co-ingestion improves post-prandial muscle protein accretion in elderly men. Clin Nutr 2013;32:412–9.
93. Casperson SL, Sheffield-Moore M, Hewlings SJ, Paddon-Jones D. Leucine supplementation chronically improves muscle protein synthesis in older adults consuming the RDA for protein. Clin Nutr 2012;31:512–9.
94. Verhoeven S, Vanschoonbeek K, Verdijk LB, Koopman R, Wodzig WK, Dendale P, van Loon LJ. Long-term leucine supplementation does not increase muscle mass or strength in healthy elderly men. Am J Clin Nutr 2009;89:1468–75.
95. Leenders M, Verdijk LB, van der Hoeven L, van Kranenburg J, Hartgens F, Wodzig WK, Saris WH, van Loon LJ. Prolonged leucine supplementation does not augment muscle mass or affect glycemic control in elderly type 2 diabetic men. J Nutr 2011;141:1070–6.
96. Engelen MP, Rutten EP, De Castro CL, Wouters EF, Schols AM, Deutz NE. Supplementation of soy protein with branched-chain amino acids alters protein metabolism in healthy elderly and even more in patients with chronic obstructive pulmonary disease. Am J Clin Nutr 2007;85:431–9.
97. Zhao W, Zhai F, Zhang D, An Y, Liu Y, He Y, Ge K, Scrimshaw NS. Lysine-fortified wheat flour improves the nutritional and immunological status of wheat-eating families in northern China. Food Nutr Bull 2004;25:123–9.
98. Hussain T, Abbas S, Khan MA, Scrimshaw NS. Lysine fortification of wheat flour improves selected indices of the nutritional status of predominantly cereal-eating families in Pakistan. Food Nutr Bull 2004;25:114–22.
99. Akalu G, Taffesse S, Gunaratna NS, De Groote H. The effectiveness of quality protein maize in improving the nutritional status of young children in the Ethiopian highlands. Food Nutr Bull 2010;31:418–30.
100. el Lozy M, Kerr GR. Results of lysine fortification of wheat products in southern Tunisia. In: Wilcke HL, editor. Improving the nutrient quality of cereals II. Report of the Second Workshop on Breeding and Fortification. Washington (DC): Agency for International Development;1976: p. 113–44.
101. Urrutia JJ, Garcia B, Bressani R, Mata L. Report of the maize fortification project in Guatemala. In: Improving the nutrient quality of cereals II. Report of the Second Workshop on Breeding and Fortification. Agency for International Development; 1976: p. 22–60.
102. Gershoff SN, McGandy RB, Suttapreyasri D, Promkutkao C, Nondasuta A, Pisolyabutra U, Tantiwongse P, Viravaidhaya V. Nutrition studied in Thailand. II. Effects of fortification of rice with lysine, threonine, thiamin, riboflavin, vitamin A, and iron on preschool children. Am J Clin Nutr 1977;30:1185–95.
103. National Research Council; Task Force on Amino Acid Fortification of CerealsThe results and interpretation of three field trials of lysine fortification of cereals. Washington (DC): National Academies Press; 1984.
104. Prasanna B, Vasal S, Kassahun B, Singh N. Quality protein maize. Curr Sci 2001;81:1308–19.
105. Tang M, He X, Luo Y, Ma L, Tang X, Huang K. Nutritional assessment of transgenic lysine-rich maize compared with conventional quality protein maize. J Sci Food Agric 2013;93:1049–54.
106. Gunaratna NS, Groote HD, Nestel P, Pixley KV, McCabe GP. A meta-analysis of community-based studies on quality protein maize. Food Policy 2010;35:202–10.
107. Reidy PT, Walker DK, Dickinson JM, Gundermann DM, Drummond MJ, Timmerman KL, Fry CS, Borack MS, Cope MB, Mukherjea R, et al. Protein blend ingestion following resistance exercise promotes human muscle protein synthesis. J Nutr 2013;143:410–6.
108. Churchward-Venne TA, Burd NA, Mitchell CJ, West DWD, Philp A, Marcotte GR, Baker SK, Baar K, Phillips SM. Supplementation of a suboptimal protein dose with leucine or essential amino acids: effects on myofibrillar protein synthesis at rest and following resistance exercise in men. J Physiol 2012;590:2751–65.