*Proceedings of the Nutrition Society* (2021), **80**, 243–251 doi:10.1017/S0029665120007880
© The Authors, 2020. Published by Cambridge University Press on behalf of The Nutrition Society.
First published online 14 October 2020

*Nutrition Society Live 2020 was held virtually on 14–15 July 2020*

## Plenary Lecture

# Protein metabolism and related body function: mechanistic approaches and health consequences

Daniel Tomé*, Simon Benoit and Dalila Azzout-Marniche
*UMR PNCA, AgroParisTech, INRAE, Université Paris-Saclay, 75005, Paris, France*

The development and maintenance of body composition and functions require an adequate protein intake with a continuous supply of amino acids (AA) to tissues. Body pool and AA cellular concentrations are tightly controlled and maintained through AA supply (dietary intake, recycled from proteolysis and *de novo* synthesis), AA disposal (protein synthesis and other AA-derived molecules) and AA losses (deamination and oxidation). Different molecular regulatory pathways are involved in the control of AA sufficiency including the mechanistic target of rapamycin complex 1, the general control non-derepressible 2/activating transcription factor 4 system or the fibroblast growth factor 21. There is a tight control of protein intake, and human subjects and animals appear capable of detecting and adapting food and protein intake and metabolism in face of foods or diets with different protein contents. A severely protein deficient diet induces lean body mass losses and ingestion of sufficient dietary energy and protein is a prerequisite for body protein synthesis and maintenance of muscle, bone and other lean tissues and functions. Maintaining adequate protein intake with age may help preserve muscle mass and strength but there is an ongoing debate as to the optimal protein intake in older adults. The protein synthesis response to protein intake can also be enhanced by prior completion of resistance exercise but this effect could be somewhat reduced in older compared to young individuals and gain in muscle mass and function due to exercise require regular training over an extended period.

**Protein: Amino acid: Regulatory pathways: Nutrient sensing: Metabolism**

A daily intake of an adequate quantity of protein from foods provides nitrogen and amino acid (AA) to support the synthesis of body proteins and as precursors of various nitrogenous and other important compounds in the body. A continuous supply of AA to tissues, and particularly essential AA (EAA) which are not *de novo* synthesised in the body, is required for survival, for the development and maintenance of body composition and to support AA-dependent metabolic processes and most if not all physiological functions. AA sufficiency in the body and in tissues and cells is tightly and continuously controlled through different sensing and signalling processes that modulate and adapt protein and energy metabolism and feeding behaviour to prevent or counteract protein deficiency and to reach and maintain a well-balanced protein status.

## Molecular mechanisms for the sensing of protein and amino acid sufficiency

AA play a central role in the metabolism and their body pool and cellular concentrations are tightly controlled and maintained through AA supply (dietary intake, recycled from proteolysis and *de novo* synthesis), AA disposal (protein synthesis and other AA-derived molecules)

**Abbreviations:** AA, amino acid; ATF4, activating transcription factor 4; EAA, essential AA; FGF21, fibroblast growth factor 21; GCN2, general control non-derepressible 2; IGF1, insulin growth factor 1; mTOR, mechanistic target of rapamycin; mTORC1, mTOR complex 1.

***Corresponding author:** Daniel Tomé, email daniel.tome@outlook.fr

https://doi.org/10.1017/S0029665120007880 Published online by Cambridge University Press

and AA losses (deamination and oxidation). At the body and cellular levels, the control and maintenance of AA sufficiency proceeds through complex sensing and signalling pathways[1]. Different mediators, hormones, signalling regulatory molecules and their upstream and downstream pathways are involved in the control and maintenance of AA sufficiency including insulin, the insulin growth factor 1 (IGF1), the fibroblast growth factor 21 (FGF21), the mechanistic target of rapamycin (mTOR) complex 1 (mTORC1), the AMP-activated protein kinase and the general control non-derepressible 2 (GCN2)/activating transcription factor 4 (ATF4) system. Numerous AA transport processes and transporters adapt to intracellular AA level and modulate cellular AA exchange with the extracellular medium through AA uptake and excretion[1–5].

The cellular availability of AA is involved in the anabolic response to feeding through mRNA translation and protein synthesis[1,6–10]. The mTOR pathway, and more precisely the mTORC1 complex constituted by the protein mTOR and the regulatory associated protein of mTOR is a central regulatory component in the sensing and signalling of cellular AA sufficiency[9,11–16]. It is a main regulator of cell growth with the capacity to phosphorylate target proteins involved in cellular anabolic pathways including protein synthesis, and in catabolic pathways including autophagy[17–19]. Under low intracellular AA concentration, mTORC1 is inactivated, leading to reduced protein synthesis and increased proteolysis through protein autophagy[19]. Under high intracellular AA concentration, mTORC1 is activated, promoting protein synthesis and inhibiting protein degradation and autophagy[7]. The active mTORC1 complex initiates mRNA translation and protein synthesis by phosphorylation of downstream target effectors including the 70-kDa ribosomal protein S6 kinase and the eukaryotic initiation factor 4E binding proteins 1 and 2[20]. The activation of mTORC1 is associated with its recruitment to the surface of the lysosome with a direct role in the control of autophagy and lysosomal biogenesis[21].

Signals that modulate mTORC1 activity and mTORC1-dependant metabolic pathways involved in the anabolic response to feeding protein, associate hormones, growth factors, increased AA concentration as precursors of protein synthesis and some specific AA also identified as signal molecules, including particularly leucine, other branched-chain AA, arginine, glutamine and lysine. Sestrin2 and CASTOR1 proteins are proposed as molecular sensors for leucine and arginine, inducing through the same cascade of cellular events mTORC1 activation[22–25]. The ingestion of protein, free branched-chain AA or free leucine, is associated with higher cellular uptake of leucine through specific transport systems, its transfer to the lysosome, the colocalisation of mTORC1 with the lysosome and the activation of mTORC1[26]. Glutamine could increase the cellular uptake of leucine through solute carriers expressed at the plasma and lysosomal membrane and also participate to induce lysosomal mTORC1 colocalisation and activation[27–29]. The ingestion of a meal containing 20–30 g leucine-rich proteins induces the activation of mTORC1 and the stimulation of skeletal muscle synthesis within 2 h, and this effect is reinforced by regular physical training[30].

The GCN2/ATF4 system controls AA insufficiency and imbalance in mammalian cells and subsequently increases the cellular AA pool by reducing translation and AA oxidation and enhancing AA uptake and biosynthesis[31–33]. In these processes, AA and insulin exert a coordinated action on translation involving mTOR, AMP-activated protein kinase and GCN2 transduction pathways and inhibition of AMP-activated protein kinase and activation of mTOR transduction pathways are required for the downregulation of the protein ubiquitination proteolytic pathway in response to high AA and insulin concentrations[34,35]. A situation of AA deficiency induces an increase in uncharged transfer ribonucleic acid that binds to GCN2 with subsequent phosphorylation of eukaryotic initiation factor-2 and the glutamate receptor 1. Interestingly, FGF21 is under the control of GCN2 that senses AA deficiency through the ATF4 pathway[36,37]. FGF21 appears as a signal of protein insufficiency and has been involved in the downstream control of metabolic processes in different organs (liver, brown adipose tissue and skeletal muscle), such as lipid oxidation, ketogenesis and glucose uptake, and in the stimulation of adaptive diet-induced thermogenesis in response to a low protein diet or to EAA restriction as shown for leucine, methionine or threonine[36,38–46]. Both energy expenditure and food intake are increased after intracerebroventricular infusion of FGF21 without affecting body composition[47,48].

## A tight control of protein intake

Subjects are able to detect and adapt food and protein intake and metabolism to maintain or restore an adequate protein status in face of different foods or diets with different protein contents classified as high (above 25–30 % energy as protein), normal (10–20 % energy), moderately restricted (5–8 % energy) or severely restricted (2–3 % energy) in protein[49]. Protein and AA sufficiency is controlled in the body including a control of the availability of the nine EAA which are not synthesised in the body and must be provided by the diet.

With an adequate or high-protein content of the diet there is no signal of AA insufficiency, and the control of food intake is mainly driven by the need for energy although conditioning and learning processes contribute to maintain a motivation for consuming protein to prevent protein deficiency. High-protein feeding usually stimulates satiety pathways by increasing anorexigenic signals and reducing both orexigenic signals and the sensitivity to feeding of reward-driven mechanisms in the brain[50–53]. This is associated with low ghrelin and high leptin plasma concentrations, low neuropeptide Y and high proopiomelanocortin levels in the hypothalamus[49,51,54–57], increased neuronal activity in the nucleus of the tractus solitary[51,58,59], reduced neuronal activity in the amygdala[60] and lower sensitivity of dopamine-dependent reward pathways to feeding food and protein[54,61–63]. This is also associated with lower

https://doi.org/10.1017/S0029665120007880 Published online by Cambridge University Press

body weight gain, and fat mass without affecting lean body mass[64–66]. Such high-protein diets have been repeatedly discussed in the context of body weight management and prevention or treatment of overweight and obesity[52,67,68]. In human subjects, a protein threshold of at least 30 g protein is required to increase fullness ratings and to elicit satiety responses compared to low-protein preloads[69,70]. Interestingly, in rats submitted to food selection with protein-rich food, there is a trend to choose a high level of protein intake that is often significantly above the protein intake required to meet protein needs derived from nitrogen balance[71–73].

With a diet marginally low in protein, the metabolic needs for protein are probably the main determinant of food intake and feeding behaviour, with different strategies such as a preference for protein-rich foods when a choice is offered or, when no food choice is allowed, hyperphagia and an increase in food intake[54,71]. Many studies indicate that, to preserve the protein intake with diets marginally reduced in protein content, subjects usually tend to eat more than a control group fed an adequate protein diet if they are not offered a choice in which protein-rich foods are included[38,74–77]. Feeding moderately deficient low-protein diet or marginally deficient in some AA more often increases orexigenic pathways and appetite and motivation for food and induces an appetite and a preference for protein-rich foods[78–80], to increase protein intake and compensate for protein and AA deficiency[75,77,81–84]. This correlates with low concentrations of leptin, insulin and IGF1, and high concentrations of ghrelin and FGF21 in the plasma with high levels of neuropeptide Y and corticotropin releasing hormone and low level of proopiomelanocortin[38,54,84–90] in the hypothalamus, and with increased sensitivity of central regions involved in reward and increased response of reward-driven mechanisms to foods, protein-rich foods[54] and savoury food cues[80]. In both animals and human subjects, a moderate protein deficiency produced by foods with a low protein content or by protein deprivation induces a specific appetite for protein[91,92].

In rats a moderately low-protein diets induces hyperphagia, but the overconsumption of food remains limited and does not allow to meet an adequate intake of protein, and therefore, body protein is decreased, whereas growth and different metabolic pathways are altered[74,75,77]. The higher energy intake also leads to a risk of excess fat deposition and body fats are increased, but this is also associated with an increase in energy expenditure that compensates in part for the overconsumption of energy and the resulting fat accumulation and adiposity[38,74,75,84,93,94]. In human subjects, energy intake is also increased with a low-protein diet with either a low-protein high-fat diet or a low-protein high-carbohydrate diet[95], and this is also associated with an increase in energy expenditure[90] that could prevent in part for the excess fat accumulation. The origin of the increase in energy expenditure in low-protein-fed subjects remains unclear and has been related to an increase in both diet-induced thermogenesis in adipose tissue, the cost of muscle activity and spontaneous activity[74,94,96–99]. It also remains unclear whether the energy expenditure in low-protein-fed subjects is secondary to the increased energy intake or if, inversely, it represents the primary specific response that is responsible for the increase in energy intake, because according to some studies, the higher energy expenditure induced by low-protein diets can occur without hyperphagia[36,100–102].

Severely deficient protein diets (2–3 % energy as protein) or devoid in one EAA usually depress food intake and induce lower weight, fat mass and lean tissues, and lower plasma protein concentrations that are characteristic of the status of protein deficiency[54,75]. When diets are severely deficient or devoid of protein or at least one EAA, both protein or AA intake cannot be efficiently increased, and this leads to metabolic imbalance that induces an aversive response to the diet by a learning process, allowing for detection and rejection of these diets[103]. A diet severely deficient in at least one EAA is rapidly rejected by rats, and the animals can recognise the missing EAA when offered a choice between different foods[104]. The deficiency is very rapidly detected within the hour following the ingestion in relation to the decrease in the corresponding EAA in the blood, leading to a rapid decrease in food intake. An incomplete protein diet is also recognised in human subjects and results in a decrease in food intake, through a signal of hunger suppression rather than satiation or satiety[105,106]. An extremely low-protein diet or diets severely deficient in at least one EAA induce imbalanced plasma and brain AA patterns, producing a conditioned taste aversion to the diet[107–109]. In this process, the decrease of the deficient EAA in the plasma, cerebrospinal fluid and brain is sensed by GCN2 that subsequently triggers a glutamatergic signalling[110]. The associated loss of γ-aminobutyric acid (GABA)ergic/inhibitory control contributes to activating glutamatergic excitatory circuits, which project to different brain regions, leading to the modification of feeding behaviour[111].

## Protein intake, protein synthesis and body composition

Protein supply is required for the development and maintenance of body composition. A balanced diet provides an adequate quantity of protein (average requirement for adults is 0·66 g/kg/d according to WHO/FAO) containing an adequate quantity of each of the nine EAA to support protein synthesis and achieve nitrogen balance.

A severely protein deficient diet induces lean body mass losses, and ingestion of sufficient dietary energy and protein is a prerequisite for body protein synthesis and maintenance of muscle, bone and other lean tissues and their functions. Proteins, the main compartment of AA in the body, are in constant turnover with free-AA through protein synthesis and degradation, and a small fraction of free-AA that is lost by oxidation in the mitochondria is replaced by AA uptake and non-EAA *de novo* synthesis. Protein synthesis, protein degradation and AA oxidation are regulated by AA availability in the cell[112–116]. In the fed state with increased

https://doi.org/10.1017/S0029665120007880 Published online by Cambridge University Press

Proceedings of the Nutrition Society

intracellular AA concentrations, protein synthesis is increased, protein degradation is reduced and AA oxidation is increased, whereas the inverse processes are induced in the fasting state. Body protein content is related to protein intake up to a plateau considered to indicate a well-balanced protein status, and above this plateau there is no more protein deposition and additional dietary AA are oxidised and lost. The exact amount of protein intake to meet the requirements for body remodelling is currently defined as the minimum amount resulting in a whole-body nitrogen and protein net balance but this remains debated due to difficulties in the identification of the more relevant markers to be used (e.g. nitrogen balance, whole-body protein mass, muscle mass, bone mass, physical function, immune function and metabolic function).

There are several mechanisms by which dietary protein improves muscle and bone mass and strength[117]. Increasing dietary protein increases circulating levels of IGF1, a key regulatory factor of bone growth but also of the skeletal response to anabolic signals, and conversely, a low-protein diet decreases IGF1[118]. Increasing the availability of AA induces an anabolic state that stimulates muscle protein synthesis and a mild suppression of protein breakdown with protein synthesis exceeding breakdown and leading to a positive protein balance[119–121]. There is a direct relationship between intracellular appearance of AA and muscle protein synthesis up to a plateau occurring with ingestion of 20–35 g high-quality protein[122–124]. However, with increasing protein ingestion, if protein synthesis rapidly increases but reaches a plateau, in contrast protein breakdown could decrease more slightly but more continuously even above the amount of protein intake at which synthesis reach a plateau. This could lead to an extra net protein balance due to decreased breakdown although this was not measured at the muscle level but mainly at the whole body level[125,126]. EAA stimulates protein synthesis while non-EAA does not have any additional effect, and the intracellular availability of EAA is the primary determinant of the rate of muscle protein synthesis[127]. EAA (especially leucine) and insulin are anabolic stimuli for muscle and share a common pathway of action via activation of mTOR[128]. The branched chain AA leucine is a key regulator of anabolic signalling in skeletal muscles and bind with Sestrin2 to induce protein synthesis[6,30,127,129–131]. However, although leucine initiates the translational processes, the other EAA are probably required to efficiently induce a protein synthesis response following protein intake in human subjects[132].

Dietary protein is important for muscle and bone development and maintenance. Short-term bed rest or disuse accelerates the loss of muscle mass, function and glucose intolerance[133]. It is known that muscle inactivity leads to loss of muscle mass, loss of muscle strength and reduced muscle oxidative capacity in human subjects[125]. Muscle protein synthesis response to protein intake is reduced in immobilised muscle and an elevated protein intake could be required to maintain whole-body postabsorptive protein turnover during inactivity[134,135]. The onset of age-related bone and muscle losses associated with osteoporosis and sarcopoenia may be increased or decreased according to lifestyle practices in early-middle age[70]. The loss of muscle and bone tends to occur at approximately the same time, and changes in muscle and bone mass are correlated[136,137]. Reduction in muscle mass and functional capacity related to ageing account for 3–8 % reduction in muscle mass per decade, starting in the fourth or fifth decade of life. There is evidence that mTORC1- and ATF4-mediated AA-sensing pathways, triggered by impaired AA delivery to skeletal muscle, reduced the anabolic responses to feeding, even if the anabolic sensitivity of tissues is not directly impaired[138,139]. Maintaining adequate protein intake with age may help to preserve muscle mass and strength[140]. There is an ongoing discussion on the optimal protein intake in older adults that could be higher than usually proposed and on the optimal feeding profile between meals[141–145]. Increasing dietary protein also resulted in lower markers of bone resorption and higher circulating levels of IGF1 in healthy older men and women[146]. Additionally, IGF1 and skeletal muscle fibre decrease in older women fed a low-protein diet, suggesting that increasing IGF1 by increasing dietary protein intake may promote muscle protein synthesis[147].

Muscle inactivity decreases and muscle activity increases muscle protein synthesis and the net balance of AA incorporated into muscle protein, but gain in muscle mass and function due to exercise require regular training over an extended period of time[125,148]. Exercise sensitises the muscle to AA and potentiates the rise in protein synthesis that, when repeated over time, results in gradual radial growth of skeletal muscle and improved muscle strength and function[149,150]. Protein supplementation during resistance exercise training increases muscle mass gain with a protein dose-dependent effect on translational regulation through mTORC1 signalling that could be enhanced by an adequate leucine intake[124,144,145,151–153]. Regular physical activity may preserve and even enhance the responsiveness of skeletal muscle to protein intake in older subjects[142]. The protein synthesis response to protein intake can also be enhanced by prior completion of resistance exercise in older subjects but this effect could be somewhat lower in older compared to young individuals[125,148,154–157].

## Conclusion

An adequate protein status is required for survival to balance nitrogen losses, for the development and maintenance of body composition, and to support most if not all physiological functions. Protein quality is based on EAA content to meet metabolic needs. The control of protein sufficiency proceeds through complex sensing and signalling pathways that control protein and food consumption and the metabolic response to feeding. Ingestion of protein promotes body protein synthesis and maintenance of lean and muscle mass and function and this effect is potentiated by exercise. These processes are particularly important to prevent lean body mass

https://doi.org/10.1017/S0029665120007880 Published online by Cambridge University Press

losses and decreased muscle strength and bone health with ageing. There is a direct relationship between intracellular appearance of AA and the rate of muscle protein synthesis. With increasing protein ingestion, body and muscle protein synthesis increases fast but reaches a plateau above which additionally provided AA are catabolised. Whether ageing is associated with diminished accretion of muscle proteins after the ingestion of protein or EAA remains under discussion. The strategies to improve protein synthesis, lean mass and muscle performance in older subjects, include per meal dose and frequency of protein consumption. The protein synthesis response to protein intake can also be enhanced by prior completion of resistance exercise but this effect could be somewhat reduced in older compared to young individuals and gain in muscle mass and function due to exercise requires regular training over an extended period.


## Financial Support

This research activity was supported by INRAE.


## Conflict of Interest

None.

## Authorship

The authors had joint responsibility for all aspects of preparation of this paper.

## References


1. Broer S & Broer A (2017) Amino acid homeostasis and signalling in mammalian cells and organisms. *Biochem J* **474**, 1935–1963.
2. Broer S & Palacin M (2011) The role of amino acid transporters in inherited and acquired diseases. *Biochem J* **436**, 193–211.
3. Hediger MA, Clemencon B, Burrier RE *et al.* (2013) The ABCs of membrane transporters in health and disease (SLC series): introduction. *Mol Aspects Med* **34**, 95–107.
4. Miller S, Chinkes D, MacLean DA *et al.* (2004) In vivo muscle amino acid transport involves two distinct processes. *Am J Physiol Endocrinol Metab* **287**, E136–E141.
5. Palacin M, Estevez R, Bertran J *et al.* (1998) Molecular biology of mammalian plasma membrane amino acid transporters. *Physiol Rev* **78**, 969–1054.
6. Anthony JC, Anthony TG, Kimball SR *et al.* (2000) Orally administered leucine stimulates protein synthesis in skeletal muscle of postabsorptive rats in association with increased eIF4F formation. *J Nutr* **130**, 139–145.
7. Kimball SR (2014) Integration of signals generated by nutrients, hormones, and exercise in skeletal muscle. *Am J Clin Nutr* **99**, 237S–242S.
8. Layman DK, Anthony TG, Rasmussen BB *et al.* (2015) Defining meal requirements for protein to optimize metabolic roles of amino acids. *Am J Clin Nutr* **101**, 1330S–1338S.
9. Li F, Yin Y, Tan B *et al.* (2011) Leucine nutrition in animals and humans: mTOR signaling and beyond. *Amino Acids* **41**, 1185–1193.
10. Lynch CJ, Halle B, Fujii H *et al.* (2003) Potential role of leucine metabolism in the leucine-signaling pathway involving mTOR. *Am J Physiol Endocrinol Metab* **285**, E854–E863.
11. Avruch J, Long X, Ortiz-Vega S *et al.* (2009) Amino acid regulation of TOR complex 1. *Am J Physiol Endocrinol Metab* **296**, E592–E602.
12. Bar-Peled L & Sabatini DM (2014) Regulation of mTORC1 by amino acids. *Trends Cell Biol* **24**, 400–406.
13. Goberdhan DC, Wilson C & Harris AL (2016) Amino acid sensing by mTORC1: intracellular transporters mark the spot. *Cell Metab* **23**, 580–589.
14. Jewell JL, Russell RC & Guan KL (2013) Amino acid signalling upstream of mTOR. *Nat Rev Mol Cell Biol* **14**, 133–139.
15. Tomé D (2018) 90th Anniversary commentary: the mTORC1 complex – a central player in the control and regulation of amino acid sufficiency. *J Nutr* **148**, 1678–1682.
16. Zheng L, Zhang W, Zhou Y *et al.* (2016) Recent advances in understanding amino acid sensing mechanisms that regulate mTORC1. *Int J Mol Sci* **17**, 1636–1651.
17. Dunlop EA & Tee AR (2014) mTOR and autophagy: a dynamic relationship governed by nutrients and energy. *Semin Cell Dev Biol* **36**, 121–129.
18. Hosokawa N, Hara T, Kaizuka T *et al.* (2009) Nutrient-dependent mTORC1 association with the ULK1-Atg13-FIP200 complex required for autophagy. *Mol Biol Cell* **20**, 1981–1991.
19. Tan VP & Miyamoto S (2016) Nutrient-sensing mTORC1: integration of metabolic and autophagic signals. *J Mol Cell Cardiol* **95**, 31–41.
20. Dennis MD, Jefferson LS & Kimball SR (2012) Role of p70S6K1-mediated phosphorylation of eIF4B and PDCD4 proteins in the regulation of protein synthesis. *J Biol Chem* **287**, 42890–42899.
21. Rabanal-Ruiz Y & Korolchuk VI (2018) mTORC1 and nutrient homeostasis: the central role of the lysosome. *Int J Mol Sci* **19**, 818–841.
22. Chantranupong L, Scaria SM, Saxton RA *et al.* (2016) The CASTOR proteins are arginine sensors for the mTORC1 pathway. *Cell* **165**, 153–164.
23. Saxton RA, Chantranupong L, Knockenhauer KE *et al.* (2016) Mechanism of arginine sensing by CASTOR1 upstream of mTORC1. *Nature* **536**, 229–233.
24. Saxton RA, Knockenhauer KE, Wolfson RL *et al.* (2016) Structural basis for leucine sensing by the Sestrin2-mTORC1 pathway. *Science* **351**, 53–58.
25. Wolfson RL, Chantranupong L, Saxton RA *et al.* (2016) Sestrin2 is a leucine sensor for the mTORC1 pathway. *Science* **351**, 43–48.
26. Sancak Y, Peterson TR, Shaul YD *et al.* (2008) The Rag GTPases bind raptor and mediate amino acid signaling to mTORC1. *Science* **320**, 1496–1501.
27. Duran RV, Oppliger W, Robitaille AM *et al.* (2012) Glutaminolysis activates Rag-mTORC1 signaling. *Mol Cell* **47**, 349–358.
28. Jewell JL, Kim YC, Russell RC *et al.* (2015) Metabolism. Differential regulation of mTORC1 by leucine and glutamine. *Science* **347**, 194–198.
29. Nicklin P, Bergman P, Zhang B *et al.* (2009) Bidirectional transport of amino acids regulates mTOR and autophagy. *Cell* **136**, 521–534.
30. Borack MS & Volpi E (2016) Efficacy and safety of leucine supplementation in the elderly. *J Nutr* **146**, 2625S–2629S.


https://doi.org/10.1017/S0029665120007880 Published online by Cambridge University Press

31. Balasubramanian MN, Butterworth EA & Kilberg MS (2013) Asparagine synthetase: regulation by cell stress and involvement in tumor biology. *Am J Physiol Endocrinol Metab* **304**, E789–E799.
32. Gallinetti J, Harputlugil E & Mitchell JR (2013) Amino acid sensing in dietary-restriction-mediated longevity: roles of signal-transducing kinases GCN2 and TOR. *Biochem J* **449**, 1–10.
33. Ye J, Kumanova M, Hart LS *et al.* (2010) The GCN2-ATF4 pathway is critical for tumour cell survival and proliferation in response to nutrient deprivation. *EMBO J* **29**, 2082–2096.
34. Chotechuang N, Azzout-Marniche D, Bos C *et al.* (2011) Down-regulation of the ubiquitin-proteasome proteolysis system by amino acids and insulin involves the adenosine monophosphate-activated protein kinase and mammalian target of rapamycin pathways in rat hepatocytes. *Amino Acids* **41**, 457–468.
35. Chotechuang N, Azzout-Marniche D, Bos C *et al.* (2009) mTOR, AMPK, and GCN2 coordinate the adaptation of hepatic energy metabolic pathways in response to protein intake in the rat. *Am J Physiol Endocrinol Metab* **297**, E1313–E1323.
36. Hill CM, Berthoud HR, Munzberg H *et al.* (2018) Homeostatic sensing of dietary protein restriction: a case for FGF21. *Front Neuroendocrinol* **51**, 125–131.
37. Hill CM, Laeger T, Dehner M *et al.* (2019) FGF21 signals protein status to the brain and adaptively regulates food choice and metabolism. *Cell Rep* **27**, 2934–2947.
38. Chalvon-Demersay T, Even PC, Tome D *et al.* (2016) Low-protein diet induces, whereas high-protein diet reduces hepatic FGF21 production in mice, but glucose and not amino acids up-regulate FGF21 in cultured hepatocytes. *J Nutr Biochem* **36**, 60–67.
39. Hotta Y, Nakamura H, Konishi M *et al.* (2009) Fibroblast growth factor 21 regulates lipolysis in white adipose tissue but is not required for ketogenesis and triglyceride clearance in liver. *Endocrinology* **150**, 4625–4633.
40. Kharitonenkov A, Shiyanova TL, Koester A *et al.* (2005) FGF-21 as a novel metabolic regulator. *J Clin Invest* **115**, 1627–1635.
41. Lees EK, Krol E, Grant L *et al.* (2014) Methionine restriction restores a younger metabolic phenotype in adult mice with alterations in fibroblast growth factor 21. *Aging Cell* **13**, 817–827.
42. Markan KR, Naber MC, Ameka MK *et al.* (2014) Circulating FGF21 is liver derived and enhances glucose uptake during refeeding and overfeeding. *Diabetes* **63**, 4057–4063.
43. Mashili FL, Austin RL, Deshmukh AS *et al.* (2011) Direct effects of FGF21 on glucose uptake in human skeletal muscle: implications for type 2 diabetes and obesity. *Diabetes Metab Res Rev* **27**, 286–297.
44. Wanders D, Forney LA, Stone KP *et al.* (2017) FGF21 mediates the thermogenic and insulin-sensitizing effects of dietary methionine restriction but not its effects on hepatic lipid metabolism. *Diabetes* **66**, 858–867.
45. Wanders D, Stone KP, Dille K *et al.* (2015) Metabolic responses to dietary leucine restriction involve remodeling of adipose tissue and enhanced hepatic insulin signaling. *Biofactors* **41**, 391–402.
46. Yap YW, Rusu PM, Chan AY *et al.* (2020) Restriction of essential amino acids dictates the systemic metabolic response to dietary protein dilution. *Nat Commun* **11**, 2894.
47. Laeger T, Henagan TM, Albarado DC *et al.* (2014) FGF21 is an endocrine signal of protein restriction. *J Clin Invest* **124**, 3913–3922.
48. Sarruf DA, Thaler JP, Morton GJ *et al.* (2010) Fibroblast growth factor 21 action in the brain increases energy expenditure and insulin sensitivity in obese rats. *Diabetes* **59**, 1817–1824.
49. Tomé D, Chaumontet C, Even PC *et al.* (2020) Protein status modulates an appetite for protein to maintain a balanced nutritional state-A perspective view. *J Agric Food Chem* **68**, 1830–1836.
50. Darcel N, Fromentin G, Raybould HE *et al.* (2005) Fos-positive neurons are increased in the nucleus of the solitary tract and decreased in the ventromedial hypothalamus and amygdala by a high-protein diet in rats. *J Nutr* **135**, 1486–1490.
51. Faipoux R, Tome D, Gougis S *et al.* (2008) Proteins activate satiety-related neuronal pathways in the brainstem and hypothalamus of rats. *J Nutr* **138**, 1172–1178.
52. Journel M, Chaumontet C, Darcel N *et al.* (2012) Brain responses to high-protein diets. *Adv Nutr* **3**, 322–329.
53. Tome D, Schwarz J, Darcel N *et al.* (2009) Protein, amino acids, vagus nerve signaling, and the brain. *Am J Clin Nutr* **90**, 838S–843S.
54. Chaumontet C, Recio I, Fromentin G *et al.* (2018) The protein status of rats affects the rewarding value of meals due to their protein content. *J Nutr* **148**, 989–998.
55. Kinzig KP, Hargrave SL, Hyun J *et al.* (2007) Energy balance and hypothalamic effects of a high-protein/low-carbohydrate diet. *Physiol Behav* **92**, 454–460.
56. Morrison CD, Xi X, White CL *et al.* (2007) Amino acids inhibit Agrp gene expression via an mTOR-dependent mechanism. *Am J Physiol Endocrinol Metab* **293**, E165–E171.
57. Zeeni N, Nadkarni N, Bell JD *et al.* (2010) Peripherally injected cholecystokinin-induced neuronal activation is modified by dietary composition in mice. *Neuroimage* **50**, 1560–1565.
58. Fromentin G, Darcel N, Chaumontet C *et al.* (2012) Peripheral and central mechanisms involved in the control of food intake by dietary amino acids and proteins. *Nutr Res Rev* **25**, 29–39.
59. Schwarz J, Burguet J, Rampin O *et al.* (2010) Three-dimensional macronutrient-associated Fos expression patterns in the mouse brainstem. *PLoS ONE* **5**, e8974.
60. Min DK, Tuor UI, Koopmans HS *et al.* (2011) Changes in differential functional magnetic resonance signals in the rodent brain elicited by mixed-nutrient or protein-enriched meals. *Gastroenterology* **141**, 1832–1841.
61. Drummen M, Dorenbos E, Vreugdenhil ACE *et al.* (2018) Associations of brain reactivity to food cues with weight loss, protein intake and dietary restraint during the PREVIEW intervention. *Nutrients* **10**, 1771–1785.
62. Leidy HJ, Lepping RJ, Savage CR *et al.* (2011) Neural responses to visual food stimuli after a normal vs. Higher protein breakfast in breakfast-skipping teens: a pilot fMRI study. *Obesity* **19**, 2019–2025.
63. Leidy HJ, Ortinau LC, Douglas SM *et al.* (2013) Beneficial effects of a higher-protein breakfast on the appetitive, hormonal, and neural signals controlling energy intake regulation in overweight/obese, 'breakfast-skipping,' late-adolescent girls. *Am J Clin Nutr* **97**, 677–688.
64. Bensaid A, Tome D, L'Heureux-Bourdon D *et al.* (2003) A high-protein diet enhances satiety without conditioned taste aversion in the rat. *Physiol Behav* **78**, 311–320.
65. Chaumontet C, Even PC, Schwarz J *et al.* (2015) High dietary protein decreases fat deposition induced by high-fat and high-sucrose diet in rats. *Br J Nutr* **114**, 1132–1142.


https://doi.org/10.1017/S0029665120007880 Published online by Cambridge University Press

66. Jean C, Rome S, Mathe V *et al.* (2001) Metabolic evidence for adaptation to a high protein diet in rats. *J Nutr* **131**, 91–98.
67. Leidy HJ, Clifton PM, Astrup A *et al.* (2015) The role of protein in weight loss and maintenance. *Am J Clin Nutr* **101**, 1320S–1329S.
68. Westerterp-Plantenga MS, Nieuwenhuizen A, Tome D *et al.* (2009) Dietary protein, weight loss, and weight maintenance. *Annu Rev Nutr* **29**, 21–41.
69. Dhillon J, Craig BA, Leidy HJ *et al.* (2016) The effects of increased protein intake on fullness: a meta-analysis and its limitations. *J Acad Nutr Diet* **116**, 968–983.
70. Paddon-Jones D & Leidy H (2014) Dietary protein and muscle in older persons. *Curr Opin Clin Nutr Metab Care* **17**, 5–11.
71. Azzout-Marniche D, Chalvon-Demersay T, Pimentel G *et al.* (2016) Obesity-prone high-fat-fed rats reduce caloric intake and adiposity and gain more fat-free mass when allowed to self-select protein from carbohydrate:fat intake. *Am J Physiol Regul Integr Comp Physiol* **310**, R1169–R1176.
72. Jean C, Fromentin G, Tome D *et al.* (2002) Wistar rats allowed to self-select macronutrients from weaning to maturity choose a high-protein, high-lipid diet. *Physiol Behav* **76**, 65–73.
73. Makarios-Lahham L, Roseau SM, Fromentin G *et al.* (2004) Rats free to select between pure protein and a fat-carbohydrate mix ingest high-protein mixed meals during the dark period and protein meals during the light period. *J Nutr* **134**, 618–624.
74. Blais A, Chaumontet C, Azzout-Marniche D *et al.* (2018) Low-protein diet-induced hyperphagia and adiposity are modulated through interactions involving thermoregulation, motor activity, and protein quality in mice. *Am J Physiol Endocrinol Metab* **314**, E139–EE51.
75. Du F, Higginbotham DA & White BD (2000) Food intake, energy balance and serum leptin concentrations in rats fed low-protein diets. *J Nutr* **130**, 514–521.
76. Leung PM & Rogers QR (1986) Effect of amino acid imbalance and deficiency on dietary choice patterns of rats. *Physiol Behav* **37**, 747–758.
77. Sorensen A, Mayntz D, Raubenheimer D *et al.* (2008) Protein-leverage in mice: the geometry of macronutrient balancing and consequences for fat deposition. *Obesity* **16**, 566–571.
78. Gibson EL & Booth DA (1986) Acquired protein appetite in rats: dependence on a protein-specific need state. *Experientia* **42**, 1003–1004.
79. Griffioen-Roose S, Mars M, Siebelink E *et al.* (2012) Protein status elicits compensatory changes in food intake and food preferences. *Am J Clin Nutr* **95**, 32–38.
80. Griffioen-Roose S, Smeets PA, van den Heuvel E *et al.* (2014) Human protein status modulates brain reward responses to food cues. *Am J Clin Nutr* **100**, 113–122.
81. Beaton JR, Feleki V & Stevenson JA (1965) Insulin hyperphagia in rats fed a low-protein diet. *Can J Physiol Pharmacol* **43**, 225–233.
82. Carreiro AL, Dhillon J, Gordon S *et al.* (2016) The macronutrients, appetite, and energy intake. *Annu Rev Nutr* **36**, 73–103.
83. White BD, Dean RG & Martin RJ (1998) An association between low levels of dietary protein, elevated NPY gene expression in the basomedial hypothalamus and increased food intake. *Nutr Neurosci* **1**, 173–182.
84. White BD, He B, Dean RG *et al.* (1994) Low protein diets increase neuropeptide Y gene expression in the basomedial hypothalamus of rats. *J Nutr* **124**, 1152–1160.
85. Chalvon-Demersay T, Even PC, Chaumontet C *et al.* (2017) Modifying the dietary carbohydrate-to-protein ratio alters the postprandial macronutrient oxidation pattern in liver of AMPK-deficient mice. *J Nutr* **147**, 1669–1676.
86. Chaumontet C, Azzout-Marniche D, Blais A *et al.* (2019) Low-protein and methionine, high-starch diets increase energy intake and expenditure, increase FGF21, decrease IGF-1, and have little effect on adiposity in mice. *Am J Physiol Regul Integr Comp Physiol* **316**, R486–R501.
87. Dickson SL, Egecioglu E, Landgren S *et al.* (2011) The role of the central ghrelin system in reward from food and chemical drugs. *Mol Cell Endocrinol* **340**, 80–87.
88. Pfaffly J, Michaelides M, Wang GJ *et al.* (2010) Leptin increases striatal dopamine D2 receptor binding in leptin-deficient obese (ob/ob) mice. *Synapse* **64**, 503–510.
89. Skibicka KP & Dickson SL (2011) Ghrelin and food reward: the story of potential underlying substrates. *Peptides* **32**, 2265–2273.
90. Vinales KL, Begaye B, Bogardus C *et al.* (2019) FGF21 is a hormonal mediator of the human 'Thrifty' metabolic phenotype. *Diabetes* **68**, 318–323.
91. Booth DA (1974) Food intake compensation for increase or decrease in the protein content of the diet. *Behav Biol* **12**, 31–40.
92. Gibson EL, Wainwright CJ & Booth DA (1995) Disguised protein in lunch after low-protein breakfast conditions food-flavor preferences dependent on recent lack of protein intake. *Physiol Behav* **58**, 363–371.
93. Donald P, Pitts CC & Pohl SL (1981) Body weight and composition in laboratory rats: effects of diets with high or low protein concentrations. *Science* **211**, 185–186.
94. Pezeshki A, Zapata RC, Singh A *et al.* (2016) Low protein diets produce divergent effects on energy balance. *Sci Rep* **6**, 25145.
95. Gosby AK, Conigrave AD, Raubenheimer D *et al.* (2014) Protein leverage and energy intake. *Obes Rev* **15**, 183–191.
96. Aparecida de Franca S, Dos Santos MP, Garofalo MA *et al.* (2009) Low protein diet changes the energetic balance and sympathetic activity in brown adipose tissue of growing rats. *Nutrition* **25**, 1186–1192.
97. Chartoumpekis DV, Habeos IG, Ziros PG *et al.* (2011) Brown adipose tissue responds to cold and adrenergic stimulation by induction of FGF21. *Mol Med* **17**, 736–740.
98. Fisher FM, Kleiner S, Douris N *et al.* (2012) FGF21 regulates PGC-1alpha and browning of white adipose tissues in adaptive thermogenesis. *Genes Dev* **26**, 271–281.
99. de Franca SA, dos Santos MP, Przygodda F *et al.* (2016) A low-protein, high-carbohydrate diet stimulates thermogenesis in the brown adipose tissue of rats via ATF-2. *Lipids* **51**, 303–310.
100. Hill CM, Laeger T, Albarado DC *et al.* (2017) Low protein-induced increases in FGF21 drive UCP1-dependent metabolic but not thermoregulatory endpoints. *Sci Rep* **7**, 8209.
101. Laeger T, Albarado DC, Burke SJ *et al.* (2016) Metabolic responses to dietary protein restriction require an increase in FGF21 that is delayed by the absence of GCN2. *Cell Rep* **16**, 707–716.
102. Rothwell NJ & Stock MJ (1987) Influence of carbohydrate and fat intake on diet-induced thermogenesis and brown fat activity in rats fed low protein diets. *J Nutr* **117**, 1721–1726.
103. Rogers QR & Leung PM (1973) The influence of amino acids on the neuroregulation of food intake. *Fed Proc* **32**, 1709–1719.

https://doi.org/10.1017/S0029665120007880 Published online by Cambridge University Press

104. Gietzen DW, Hao S & Anthony TG (2007) Mechanisms of food intake repression in indispensable amino acid deficiency. *Annu Rev Nutr* **27**, 63–78.
105. Nieuwenhuizen AG, Hochstenbach-Waelen A, Veldhorst MA *et al.* (2009) Acute effects of breakfasts containing alpha-lactalbumin, or gelatin with or without added tryptophan, on hunger, 'satiety' hormones and amino acid profiles. *Br J Nutr* **101**, 1859–1866.
106. Veldhorst MA, Nieuwenhuizen AG, Hochstenbach-Waelen A *et al.* (2009) A breakfast with alpha-lactalbumin, gelatin, or gelatin + TRP lowers energy intake at lunch compared with a breakfast with casein, soy, whey, or whey-GMP. *Clin Nutr* **28**, 147–155.
107. Even PC, Rolland V, Feurte S *et al.* (2000) Postprandial metabolism and aversive response in rats fed a threonine-devoid diet. *Am J Physiol Regul Integr Comp Physiol* **279**, R248–R254.
108. Feurte S, Nicolaidis S, Even PC *et al.* (1999) Rapid fall in plasma threonine followed by increased intermeal interval in response to first ingestion of a threonine-devoid diet in rats. *Appetite* **33**, 329–341.
109. Feurte S, Tome D, Gietzen DW *et al.* (2002) Feeding patterns and meal microstructure during development of a taste aversion to a threonine devoid diet. *Nutr Neurosci* **5**, 269–278.
110. Hao S, Sharp JW, Ross-Inta CM *et al.* (2005) Uncharged tRNA and sensing of amino acid deficiency in mammalian piriform cortex. *Science* **307**, 1776–1778.
111. Anthony TG & Gietzen DW (2013) Detection of amino acid deprivation in the central nervous system. *Curr Opin Clin Nutr Metab Care* **16**, 96–101.
112. Hinnebusch AG, Ivanov IP & Sonenberg N (2016) Translational control by 5′-untranslated regions of eukaryotic mRNAs. *Science* **352**, 1413–1416.
113. Hinnebusch AG (1994) Translational control of GCN4: an in vivo barometer of initiation-factor activity. *Trends Biochem Sci* **19**, 409–414.
114. Hinnebusch AG (1994) The eIF-2 alpha kinases: regulators of protein synthesis in starvation and stress. *Semin Cell Biol* **5**, 417–426.
115. Kimball SR & Jefferson LS (2005) Role of amino acids in the translational control of protein synthesis in mammals. *Semin Cell Dev Biol* **16**, 21–27.
116. Kimball SR & Jefferson LS (2010) Control of translation initiation through integration of signals generated by hormones, nutrients, and exercise. *J Biol Chem* **285**, 29027–29032.
117. Gaffney-Stomberg E, Insogna KL, Rodriguez NR *et al.* (2009) Increasing dietary protein requirements in elderly people for optimal muscle and bone health. *J Am Geriatr Soc* **57**, 1073–1079.
118. Bonjour JP, Schurch MA, Chevalley T *et al.* (1997) Protein intake, IGF-1 and osteoporosis. *Osteoporos Int* **7**, Suppl. 3, S36–S42.
119. Biolo G, Tipton KD, Klein S *et al.* (1997) An abundant supply of amino acids enhances the metabolic effect of exercise on muscle protein. *Am J Physiol* **273**, E122–E129.
120. Kobayashi H, Borsheim E, Anthony TG *et al.* (2003) Reduced amino acid availability inhibits muscle protein synthesis and decreases activity of initiation factor eIF2B. *Am J Physiol Endocrinol Metab* **284**, E488–E498.
121. Wolfe RR (2018) The 2017 Sir David P Cuthbertson lecture. Amino acids and muscle protein metabolism in critical care. *Clin Nutr* **37**, 1093–1100.
122. Deutz NE & Wolfe RR (2013) Is there a maximal anabolic response to protein intake with a meal? *Clin Nutr* **32**, 309–313.
123. Moore DR, Robinson MJ, Fry JL *et al.* (2009) Ingested protein dose response of muscle and albumin protein synthesis after resistance exercise in young men. *Am J Clin Nutr* **89**, 161–168.
124. Witard OC, Jackman SR, Breen L *et al.* (2014) 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* **99**, 86–95.
125. Dideriksen K, Reitelseder S & Holm L (2013) Influence of amino acids, dietary protein, and physical activity on muscle mass development in humans. *Nutrients* **5**, 852–876.
126. Kim IY, Schutzler S, Schrader A *et al.* (2016) The anabolic response to a meal containing different amounts of protein is not limited by the maximal stimulation of protein synthesis in healthy young adults. *Am J Physiol Endocrinol Metab* **310**, E73–E80.
127. Volpi E, Kobayashi H, Sheffield-Moore M *et al.* (2003) Essential amino acids are primarily responsible for the amino acid stimulation of muscle protein anabolism in healthy elderly adults. *Am J Clin Nutr* **78**, 250–258.
128. Wullschleger S, Loewith R & Hall MN (2006) TOR signaling in growth and metabolism. *Cell* **124**, 471–484.
129. Anthony JC, Yoshizawa F, Anthony TG *et al.* (2000) Leucine stimulates translation initiation in skeletal muscle of postabsorptive rats via a rapamycin-sensitive pathway. *J Nutr* **130**, 2413–2419.
130. Casperson SL, Sheffield-Moore M, Hewlings SJ *et al.* (2012) Leucine supplementation chronically improves muscle protein synthesis in older adults consuming the RDA for protein. *Clin Nutr* **31**, 512–519.
131. Glynn EL, Fry CS, Drummond MJ *et al.* (2010) Excess leucine intake enhances muscle anabolic signaling but not net protein anabolism in young men and women. *J Nutr* **140**, 1970–1976.
132. Wolfe RR (2017) Branched-chain amino acids and muscle protein synthesis in humans: myth or reality? *J Int Soc Sports Nutr* **14**, 30.
133. Galvan E, Arentson-Lantz E, Lamon S *et al.* (2016) Protecting skeletal muscle with protein and amino acid during periods of disuse. *Nutrients* **8**, 414–415.
134. Biolo G, Ciocchi B, Lebenstedt M *et al.* (2004) Short-term bed rest impairs amino acid-induced protein anabolism in humans. *J Physiol* **558**, 381–388.
135. Stuart CA, Shangraw RE, Peters EJ *et al.* (1990) Effect of dietary protein on bed-rest-related changes in whole-body-protein synthesis. *Am J Clin Nutr* **52**, 509–514.
136. Cohn SH, Vaswani A, Zanzi I *et al.* (1976) Changes in body chemical composition with age measured by total-body neutron activation. *Metabolism* **25**, 85–95.
137. Frost HM (1997) On our age-related bone loss: insights from a new paradigm. *J Bone Miner Res* **12**, 1539–1546.
138. Kramer IF, Verdijk LB, Hamer HM *et al.* (2017) Both basal and post-prandial muscle protein synthesis rates, following the ingestion of a leucine-enriched whey protein supplement, are not impaired in sarcopenic older males. *Clin Nutr* **36**, 1440–1449.
139. Moro T, Ebert SM, Adams CM *et al.* (2016) Amino acid sensing in skeletal muscle. *Trends Endocrinol Metab* **27**, 796–806.
140. Sahni S, Mangano KM, Hannan MT *et al.* (2015) Higher protein intake is associated with higher lean mass and quadriceps muscle strength in adult men and women. *J Nutr* **145**, 1569–1575.
141. Devries MC, McGlory C, Bolster DR *et al.* (2018) Leucine, not total protein, content of a supplement is the primary determinant of muscle protein anabolic responses in healthy older women. *J Nutr* **148**, 1088–1095.

Proceedings of the Nutrition Society

https://doi.org/10.1017/S0029665120007880 Published online by Cambridge University Press

142. Franzke B, Neubauer O, Cameron-Smith D *et al.* (2018) Dietary protein, muscle and physical function in the very old. *Nutrients* **10**, 935–949.
143. Kim IY, Schutzler S, Schrader AM *et al.* (2018) Protein intake distribution pattern does not affect anabolic response, lean body mass, muscle strength or function over 8 weeks in older adults: a randomized-controlled trial. *Clin Nutr* **37**, 488–493.
144. Phillips SM (2016) The impact of protein quality on the promotion of resistance exercise-induced changes in muscle mass. *Nutr Metab* **13**, 64.
145. Phillips SM, Chevalier S & Leidy HJ (2016) Protein 'requirements' beyond the RDA: implications for optimizing health. *Appl Physiol Nutr Metab* **41**, 565–572.
146. Dawson-Hughes B, Harris SS, Rasmussen H *et al.* (2004) Effect of dietary protein supplements on calcium excretion in healthy older men and women. *J Clin Endocrinol Metab* **89**, 1169–1173.
147. Castaneda C, Gordon PL, Fielding RA *et al.* (2000) Marginal protein intake results in reduced plasma IGF-I levels and skeletal muscle fiber atrophy in elderly women. *J Nutr Health Aging* **4**, 85–90.
148. Cermak NM, Res PT, de Groot LC *et al.* (2012) Protein supplementation augments the adaptive response of skeletal muscle to resistance-type exercise training: a meta-analysis. *Am J Clin Nutr* **96**, 1454–1464.
149. Loenneke JP, Loprinzi PD, Murphy CH *et al.* (2016) Per meal dose and frequency of protein consumption is associated with lean mass and muscle performance. *Clin Nutr* **35**, 1506–1511.
150. Stokes T, Hector AJ, Morton RW *et al.* (2018) Recent perspectives regarding the role of dietary protein for the promotion of muscle hypertrophy with resistance exercise training. *Nutrients* **10**, 180–198.
151. Davies RW, Carson BP & Jakeman PM (2018) The effect of whey protein supplementation on the temporal recovery of muscle function following resistance training: a systematic review and meta-analysis. *Nutrients* **10**, 221–231.
152. Reidy PT & Rasmussen BB (2016) Role of ingested amino acids and protein in the promotion of resistance exercise-induced muscle protein anabolism. *J Nutr* **146**, 155–183.
153. Schoenfeld BJ & Aragon AA (2018) How much protein can the body use in a single meal for muscle-building? Implications for daily protein distribution. *J Int Soc Sports Nutr* **15**, 10.
154. Moro T, Brightwell CR, Deer RR *et al.* (2018) Muscle protein anabolic resistance to essential amino acids does not occur in healthy older adults before or after resistance exercise training. *J Nutr* **148**, 900–909.
155. Morton RW, Murphy KT, McKellar SR *et al.* (2018) A systematic review, meta-analysis and meta-regression of the effect of protein supplementation on resistance training-induced gains in muscle mass and strength in healthy adults. *Br J Sports Med* **52**, 376–384.
156. Pennings B, Koopman R, Beelen M *et al.* (2011) 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* **93**, 322–331.
157. Shad BJ, Thompson JL & Breen L (2016) Does the muscle protein synthetic response to exercise and amino acid-based nutrition diminish with advancing age? A systematic review. *Am J Physiol Endocrinol Metab* **311**, E803–EE17.

Proceedings of the Nutrition Society

https://doi.org/10.1017/S0029665120007880 Published online by Cambridge University Press