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## Plenary Lecture

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

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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.

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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<sup>(1)</sup>. 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<sup>(1–5)</sup>.

The cellular availability of AA is involved in the anabolic response to feeding through mRNA translation and protein synthesis<sup>(1,6–10)</sup>. 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<sup>(9,11–16)</sup>. 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<sup>(17–19)</sup>. Under low intracellular AA concentration, mTORC1 is inactivated, leading to reduced protein synthesis and increased proteolysis through protein autophagy<sup>(19)</sup>. Under high intracellular AA concentration, mTORC1 is activated, promoting protein synthesis and inhibiting protein degradation and autophagy<sup>(7)</sup>. 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<sup>(20)</sup>. 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<sup>(21)</sup>.

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<sup>(22–25)</sup>. 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<sup>(26)</sup>. 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<sup>(27–29)</sup>. 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<sup>(30)</sup>.

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<sup>(31–33)</sup>. 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<sup>(34,35)</sup>. 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<sup>(36,37)</sup>. 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<sup>(36,38–46)</sup>. Both energy expenditure and food intake are increased after intracerebroventricular infusion of FGF21 without affecting body composition<sup>(47,48)</sup>.

### 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<sup>(49)</sup>. 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<sup>(50–53)</sup>. This is associated with low ghrelin and high leptin plasma concentrations, low neuropeptide Y and high proopiomelanocortin levels in the hypothalamus<sup>(49,51,54–57)</sup>, increased neuronal activity in the nucleus of the tractus solitarius<sup>(51,58,59)</sup>, reduced neuronal activity in the amygdala<sup>(60)</sup> and lower sensitivity of dopamine-dependent reward pathways to feeding food and protein<sup>(54,61–63)</sup>. This is also associated with lower



body weight gain, and fat mass without affecting lean body mass<sup>(64–66)</sup>. Such high-protein diets have been repeatedly discussed in the context of body weight management and prevention or treatment of overweight and obesity<sup>(52,67,68)</sup>. 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<sup>(69,70)</sup>. 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<sup>(71–73)</sup>.

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<sup>(54,71)</sup>. 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<sup>(38,74–77)</sup>. 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<sup>(78–80)</sup>, to increase protein intake and compensate for protein and AA deficiency<sup>(75,77,81–84)</sup>. 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<sup>(38,54,84–90)</sup> 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<sup>(54)</sup> and savoury food cues<sup>(80)</sup>. 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<sup>(91,92)</sup>.

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<sup>(74,75,77)</sup>. 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<sup>(38,74,75,84,93,94)</sup>. 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<sup>(95)</sup>, and this is also associated with an increase in energy expenditure<sup>(90)</sup> 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<sup>(74,94,96–99)</sup>.

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<sup>(36,100–102)</sup>.

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<sup>(54,75)</sup>. 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<sup>(103)</sup>. 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<sup>(104)</sup>. 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<sup>(105,106)</sup>. 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<sup>(107–109)</sup>. 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<sup>(110)</sup>. The associated loss of  $\gamma$ -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<sup>(111)</sup>.

### 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<sup>(112–116)</sup>. In the fed state with increased

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<sup>(117)</sup>. 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<sup>(118)</sup>. 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<sup>(119–121)</sup>. 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<sup>(122–124)</sup>. 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<sup>(125,126)</sup>. 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<sup>(127)</sup>. EAA (especially leucine) and insulin are anabolic stimuli for muscle and share a common pathway of action via activation of mTOR<sup>(128)</sup>. The branched chain AA leucine is a key regulator of anabolic signalling in skeletal muscles and bind with Sestrin2 to induce protein synthesis<sup>(6,30,127,129–131)</sup>. 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<sup>(132)</sup>.

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<sup>(133)</sup>. It is known that muscle inactivity leads to loss of muscle mass, loss of muscle strength and reduced muscle oxidative capacity in human subjects<sup>(125)</sup>. 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<sup>(134,135)</sup>. The onset of age-related bone and muscle losses

associated with osteoporosis and sarcopenia may be increased or decreased according to lifestyle practices in early-middle age<sup>(70)</sup>. The loss of muscle and bone tends to occur at approximately the same time, and changes in muscle and bone mass are correlated<sup>(136,137)</sup>. 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<sup>(138,139)</sup>. Maintaining adequate protein intake with age may help to preserve muscle mass and strength<sup>(140)</sup>. 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<sup>(141–145)</sup>. Increasing dietary protein also resulted in lower markers of bone resorption and higher circulating levels of IGF1 in healthy older men and women<sup>(146)</sup>. 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<sup>(147)</sup>.

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<sup>(125,148)</sup>. 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<sup>(149,150)</sup>. 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<sup>(124,144,145,151–153)</sup>. Regular physical activity may preserve and even enhance the responsiveness of skeletal muscle to protein intake in older subjects<sup>(142)</sup>. 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<sup>(125,148,154–157)</sup>.

## 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

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.

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### Conflict of Interest

None.

### Authorship

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

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