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PERSPECTIVE

Protein: what's on in research on clinical nutrition

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Introduction

Proteins are dietary components indispensable for maintenance and survival. Proteins contribute to nutritional requirements through the provision of nitrogen and amino acids (AAs), which are converted according to the metabolic needs into body proteins and various biochemical intermediates involved in different cellular and body activities, metabolism and functions. The amount (and quality) of protein intake and the dietary protein role in healthy subjects and in different clinical nutritional conditions and physio-pathological perspectives are widely debated.

Basics in clinical nutrition

Systemic AA homeostasis is tightly regulated, resulting in constant plasma and cellular levels [1]. Body protein and AA metabolism proceeds by a continuous turnover of body protein and free AA pools through protein synthesis and degradation, AA degradation and losses through metabolic and catabolic pathways, and AA supply through de novo synthesis and dietary intake [2]. AAs are the substrates of protein synthesis and are also the main nitrogen-containing precursors for nitrogen-containing molecules, including de novo synthesis of dispensable AAs, hormones, neurotransmitters and specialised metabolites (i.e., glutamate, serotonin, polyamines, creatine, phosphatidylserine and nitric oxide), and purine and pyrimidine nucleotides for the synthesis of nucleic acids (i.e., DNA/RNA) and energy transfer intermediates (i.e., ATP, ADP and IMP). In addition, after deamination AAs provide carbon skeletons, which are substrates for gluconeogenesis, for production of intermediates of the TCA cycle, for production of C1 carbon intermediates and as energy substrate with an energy value equivalent to glucose.

The 20 proteinogenic AAs are the precursors of protein synthesis. Protein synthesis and degradation are major factors in AA homeostasis and are tightly regulated by AA availability and energy to sustain the metabolic processes [1–4]. Whole-body proteins account for about 10 kg in male adult with a turnover of about 250–300 g per day and muscle is the largest protein compartment. In the post-absorptive state subjects are catabolic and in negative net protein balance with protein breakdown exceeding protein synthesis while feeding stimulates protein synthesis and net deposition in muscle and other tissues [1, 5, 6]. Insulin promotes AA uptake, particularly in muscle, and muscle protein synthesis is promoted by AAs, particularly leucine, insulin and insulin-like growth factor-1 [3, 6–8]. Synthesis of albumin and other protein in the liver is also regulated by AA availability. In the transition from the fed to the post-absorptive state, protein degradation increases to a level related to habitual protein intake. Autophagy plays a significant role in the maintenance of plasma AA concentrations under starvation conditions. AAs regulate systemic autophagy, particularly in the liver and less in muscle where insulin is the main regulator [9]. In healthy subject with very low protein intake for several days, nitrogen losses decrease from about 1 to 0.4 g/kg/day with a down-regulation of liver AA oxidation.

AA supply is supported by dietary intake and de novo synthesis of dispensable AA [1, 2, 10]. In the intestinal lumen, dietary protein and luminaly secreted endogenous protein are digested by gastric, pancreatic and intestinal epithelial cell proteases and peptidases, and released to the portal and peripheral circulation as AAs used by tissue with many transport activities that control their entry and exit in cells [10, 11]. Postprandial AA concentration is slightly increase but this is only significantly with high protein intake. The liver plays an important role in buffering peripheral blood AA level, as this organ metabolises about

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60% of portal blood AAs, but with important differences between AAs, with alanine and glutamine being extensively metabolised and branched-chain AAs (BCAAs) and glutamate poorly removed by the liver. In addition, de novo synthesis of dispensable AAs proceeds in a variety of cell types through an active process that involves the transfer of nitrogen between AAs. There is also an active AA reabsorption in the kidney through AA and peptide transport systems to recycle and spare AAs.

Recommendations for protein reference intakes in healthy adults at maintenance are based on nitrogen balance, which reflect the minimum amount of nitrogen intake required to balance nitrogen losses under conditions of energy balance. This leads to a mean requirement of 0.66 g/kg bodyweight (BW)/day of good-quality protein, and a recommended reference intake for young and older adults of 0.83 g protein/kg BW/day [2]. A factorial approach considering a maintenance component and an additional component of net protein deposition for growth, gestation or lactation is used for infant and children, and for pregnant and lactating women, respectively [2]. The 20 proteinogenic AAs are the precursors of protein synthesis and if non-dietary essential (dispensable) AAs can be synthesised by many cells from nitrogen and metabolic intermediates, dietary essential (or indispensable) AAs that must be acquired from dietary origin are limiting factor for protein synthesis and their availability controls protein turnover and homeostasis. Indispensable AA content is the main criteria for assessing protein quality and the currently accepted method is a chemical scoring that relates the indispensable AA content corrected by their bioavailability of individual foodstuffs or diets to reference indispensable AA profiles [2, 12–15].

Present research activities

Important questions are progressively understood in the relation between protein and AA metabolism and energy metabolism. Under nutrient sufficiency in healthy adults, there is a constant turnover of proteins with most AAs being recycled over time, and net losses are mainly related to AA oxidation in the mitochondria. Unavoidable losses of 20–25 g of protein/day are replaced by de novo synthesis and dietary intake. AAs can be oxidised in many cells in the mitochondria and the AA-derived carbon skeleton can be used by the liver to produce glucose or as substrates in tissues fuelling the Kreb's cycle with an energy content equivalent to that of carbohydrates (16–17 kJ/g) [16, 17]. Short-term fasting does not significantly decrease plasma AA except for alanine used for gluconeogenesis. Alanine is the main final product of protein degradation in muscle where upon stress and nutrient limitation glucocorticoids both inhibit protein synthesis and increase the release of alanine. During prolonged fasting glucagon increases gluconeogenesis, and blood plasma AA concentration decreases, more particularly for alanine, citrulline, proline, ornithine, tyrosine, glycine and threonine.

Muscle protein synthesis in older adults and in chronic disease is an important topic of research in the last decades. In older adults the anabolic resistance of muscle protein synthesis to nutritional stimulation by protein and AA intake, particularly at low doses, leads to muscle wasting and lower muscle strength (sarcopenia) [5, 18, 19]. In addition, patients with chronic disease often show impaired protein and AA metabolism with increased morbidity and mortality [20–23]. Muscle proteins and circulating liver-synthesised proteins are the main body stores for the supply of free AAs either recycled for protein synthesis or metabolised in different metabolic and catabolic pathways [21, 24]. For both older subjects and patients with chronic diseases the balance between anabolic and catabolic stimulation can be altered with an increased catabolic state in relation with a modified pattern of anabolic (e.g., insulin, IGFs and growth hormone) and catabolic (tumour nerosis factor-alpha, cortisol, catecholamines, glucagons and cytokines) circulating mediators [22, 23]. This catabolic state also induces insulin resistance that impairs protein and AA metabolism [25].

Research is also conducted for a better understanding of the regulatory mechanisms that ensure a homeostatic intra- and extracellular AA composition. AAs are important potential signals to modulate protein turnover and AA metabolism [26]. The serine/threonine kinase mechanistic target of rapamycin (mTOR), and more precisely the mTORC1 complex, is identified as the main AA sensor for maintaining protein and AA homeostasis [27–30]. This mTORC1 complex regulates protein translation through its downstream effector p70S6 kinase and direct target 4E-binding protein 1, and autophagy through interaction with the UNC51-like kinase 1 (ulk1)/autophagy 13 (atg13)/focal adhesion kinase-interacting protein 200 kDa (FIP200) complex [31–34]. An anabolic stimulus such as BCAAs or leucine alone shifts protein net balance from catabolism to anabolism by activation of mTORC1 in the presence of insulin, promoting protein biosynthesis and reducing autophagy [3, 35, 36]. Under conditions of AA depletion, mTORC1 is inactivated and this reduces protein synthesis and increases protein breakdown and autophagy.

In addition, while mTORC1 senses AA sufficiency, other regulatory pathways are involved in the sensing of AA deficiency. Many pathways are upregulated by transcription factor ATF4, which is induced upon AA limitation. The general control nonderepressible 2 (GCN2)/ATF4 system in mammalian cells senses AA insufficiency and imbalance and subsequently increases the cellular AA pool by

reducing translation and AA oxidation and enhancing AA uptake and biosynthesis [37–39]. In addition, AAs and insulin exert a coordinated action on translation involving mTOR, AMPK and GCN2 transduction pathways [40], and the inhibition of AMPK and the activation of mTOR transduction pathways are required for the downregulation of protein ubiquitination in response to high AA and insulin concentrations [41]. In contrast, FGF21, initially identified as sensitive to AA, mainly appears as glucose-sensitive [42].

Need of future research

The mTORC1 complex appears as a main regulator of cell growth in eukaryotic cells. The active mTORC1 promotes cellular anabolic processes, including protein, pyrimidine and lipid biosynthesis, relevant to cell growth and proliferation, and inhibits catabolic processes such as autophagy and has been associated to different physiopathological processes related to important health problems, including diabetes, cancer or neurodegenerative disorders [5, 43, 44]. Different still incompletely understood questions are related to the mechanisms by which BCAA but also other AAs such as arginine, glutamine or lysine are sensed and lead to the activation of mTORC1 [45–47], to the role of cytoplasmic structures other than the lysosome [48–50] and to the downstream pathways controlled by mTORC1 in the different cells, tissues and organs [51]. The lysosome is identified as an important structure in sensing AA availability by a mechanism involving the vacuolar ATPase (v-ATPase) and signalling pathways involved in cell metabolism and growth [5, 43, 44, 52–54]. After ingestion of protein, BCAAs or isolated leucine, leucine reaches the cell, moves into the lysosome, initiates the colocalization of the lysosome with mTORC1, and the lysosomal membrane protein v-ATPase transduces the signal to the Rag GTPases that induces the binding of the Ragulator proteins to mTORC1 [5, 48, 55]. With low AA concentration the GTPase-activating protein activity towards Rags (GATOR) 1 protein acts as a negative regulator of AA sensing while with high AA availability GATOR2 inhibits GATOR1, and the Ras homologue enriched in brain (Rheb) protein binds to the catalytic domain of mTORC1 and initiates mTOR signalling and phosphorylation of downstream effectors [56–58].

The amount and AA pattern of proteins being turned over within the body and the needs for net deposition characterise the pattern that must be made available. The amount and pattern varies with age, composition of tissue deposition and recovery of functional competence, and determine the quantitative and qualitative requirement to achieve protein and nitrogen balance [59]. Recommendations for higher protein intake of 1–1.6 g/kg/day are discussed considering biomarkers other than nitrogen balance and related to muscle mass, muscle protein synthesis and muscle strength and function or to overweight, obesity, diabetes and cardiometabolic risk through modulations in fat and glucose metabolism, energy metabolism and energy intake [36, 60–71]. In addition to total protein, the amount of protein consumed at each meal to achieve optimal (muscle) protein synthetic rates and metabolic responses is also discussed in a range of 20–35 g/meal in non-exercising or exercising young and older subjects [26, 36, 63, 72]. Interestingly, mTORC1 leads to the stimulation of skeletal muscle synthesis, preferentially within 2 h after the ingestion of a meal containing at least 20–30 g leucine-rich proteins [73–75]. In older adults, protein intakes at higher level (1.0–1.5 g/kg/day) and/or equally distributed in the different meals (20–30 g protein/meal) and with high content of BCAA or leucine (2–4 g/meal) remain discussed with contrasted results to stimulate muscle protein synthesis and maintaining muscle mass and functions or attenuating sarcopenia [36, 76–86].

The amount and composition of protein required to maintain nitrogen balance and protein and AA homeostasis, to restore and maintain function, and to limit loss of lean tissue in older subjects and in patients with syndromes characterised by protein disarrangement may differ substantially from that in healthy subjects. AA signalling influences different signalling regulatory systems, including mTORC1, GCN2 and numerous neuropeptides and hormones (i.e., GLP-1, PYY, serotonin and insulin) involved in the control of protein synthesis and other processes, including mitochondrial activity, response to inflammation or feeding involves, and the sensitivity to these metabolic signals seems to decline with age. It is discussed to increase the recommended protein intake to 1.0–1.5 g/kg/day in some physio-pathological conditions and even to 2.0 g/kg/day for older people with severe illnesses ([26, 87–90]. In addition to protein intake, in older and unhealthy subjects a different pattern of indispensable AA can be required to support the synthesis of some specific protein or metabolites, including for instance proline for collagen synthesis, aromatic AAs for synthesis of acute phase proteins and some dispensable AA can become conditionally indispensable, such as cysteine for the synthesis of glutathione or glutamine for rapidly dividing cells [26, 91, 92].

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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