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The Biological Value of Protein

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Abstract

The biological value of a protein extends beyond its amino-acid composition and digestibility, and can be influenced by additional factors in a tissue-specific manner. In healthy individuals, the slow appearance of dietary amino acids in the portal vein and subsequently in the systemic circulation in response to bolus protein ingestion improves nitrogen retention and decreases urea production. This is promoted by slow absorption when only protein is ingested (e.g. casein). When a full meal is ingested, whey achieves slightly better nitrogen retention than soy or casein, which is very likely achieved by its high content of essential amino acids (especially leucine). Elderly people exhibit 'anabolic resistance' implying that more protein is required to reach maximal rates of muscle protein synthesis compared to young individuals. Protein utilization in inflammatory or traumatic conditions increases substantially in the splanchnic tissues containing most of the immune system, and in wounds and growing tissues. This happens especially in the elderly, which often suffer from chronic inflammatory activity due to disease, physical inactivity and/or the aging process itself. Consequently, the proportion of protein absorbed in the gut and utilized for muscle protein synthesis decreases in these situations. This compromises dietary-protein-induced stimulation of muscle protein synthesis and ultimately results in increased requirements of protein (~1.2 g/kg body weight/day) to limit gradual muscle loss with age. To optimally preserve muscle mass, physical exercise is required. Exercise has both direct effects on muscle mass and health, and indirect effects by increasing the utilization of dietary protein (especially whey) to enhance rates of muscle protein synthesis.

Introduction

There is still much debate regarding the daily requirements of protein, and consensus conferences have yielded varying results. A general recommendation is to include 0.8 g of protein per kilogram of body weight in adults per day in the diet, although recent reevaluation of protein requirements with stable isotopes (as compared to the nitrogen balance methodology) suggests that this recommendation is an underestimate [1, 2]. The criticisms regarding the old recommendations are based on the observation that with increasing protein intake, a positive nitrogen balance is generally overestimated leading to an underestimation of protein requirements, which should therefore be 20–30% higher than has been believed. Similarly, defining the biological value of a protein has been subject to long-term debate. One of the problems of proteins is that their actual absorption and incorporation into body proteins has been claimed to be subject to error because it cannot be excluded that amino nitrogen is lost in the stools or metabolized by the bacterial flora. Moreover, the way in which protein quality is scored is an additional point of debate [3] as the biological effect of different proteins in different tissues of the body (e.g. skeletal muscle) is not always consistent despite apparently equivalent biological values [4]. Requirements in absolute numbers/weight/day have generally been defined without taking the different factors that may influence these requirements into account. In part, this is understandable, because this practice has been applied to deliver clear messages to the (political) community regarding protein requirements on a population basis.

Some factors that can influence requirements include the total composition of the protein-containing meal, the specific protein included, the rate of absorption of the protein as amino acids or oligopeptides, their use for incorporation into body proteins, the distribution of the meals, physical activity, the influence of aging and disease, the role of specific amino acids and the presence of growth factors in dietary protein.

The primary purpose of this chapter is to highlight the influence that some of these factors have on muscular mass and function, and to recommend dietary practices to optimize protein accrual and fitness.

Biological Value of Proteins

The biological value of a protein has traditionally been defined on the basis of its amino-acid content in relation to human requirements and the suitability for digestion, absorption and incorporation into body proteins. Much debate regarding the method to be used revolves around the question of how much of the protein is actually absorbed as intact amino acids and utilized for the synthesis of protein, bases of nucleotides and other less abundant products. The focus of this debate has especially been to arrive at recommendations for daily protein requirements independent of other factors that may contribute to adequate utilization of dietary protein. The role of energy in the meal, amounts and amino acid composition of protein, meal distribution over the day and effects of physical activity are important factors determining protein requirements. Moreover, the biological end point and potential tissue of interest (e.g. whole-body protein vs. muscle protein) should be considered when evaluating the biological value or 'quality' of a protein for a given population.

Absorption of Protein and Nitrogen Retention

It is well known that the inclusion of oxidizable fuel to a protein meal promotes nitrogen retention. Carbohydrates specifically promote net protein synthesis at the whole-body level (especially when assessed through nitrogen metabolism) primarily due to a reduction in whole-body protein breakdown. Meals completely devoid of glucose need gluconeogenesis from amino acids and to a lesser extent from glycerol to furnish the necessary glucose, which is required for maintenance of the redox state and synthesis of cell elements like membranes, bases, ribose and matrix. Consequently, a meal without carbohydrates but adequate in (fat) calories and protein promotes less nitrogen retention than a meal containing carbohydrate and protein [5]. This is even truer when cell proliferation is stimulated in inflammatory states or in states of growth. In a study in multicatheterized pigs, we found that the addition of maltodextrin to a protein meal diminished urea production compared to pigs only receiving protein [5]. Simultaneously, it was shown that amino acids appeared less abundantly in the portal vein when carbohydrates where added to the meal but that appearance was more prolonged, whereas the area under the curve (representing total uptake of protein-derived amino acids) was similar in both groups. Explanations for these findings include a slower absorption when maltodextrin was added or temporary retention of amino acids in the gut lumen as bacterial protein or inside the portal-drained viscera as rapidly turning over protein [5]. These rapidly turning over proteins should then be synthesized after mucosal absorption of amino acids after digestion of meal-derived protein. It is to our knowledge not precisely established if (and to what extent) dietary-protein-derived oligopeptides are released in the portal vein.

In another in vivo pig experiment, the absorption kinetics of different proteins were studied. Casein-derived amino-acid appearance in the portal vein proved to be less pronounced but more protracted than soy-protein-derived amino acids, which was associated with a greater nitrogen retention [6]. Comparable findings were reported by another group in human studies employing ^{13}C-leucine-labeled cow's milk, showing that whey-protein-derived amino acids were absorbed faster than casein but that whole-body nonoxidative leucine retention (a surrogate marker of protein synthesis that represents the net sum of all anabolic protein pathways in the body, such as splanchnic, kidney, muscle and circulatory) was lower in the whey group than in the casein group in healthy young men [7]. Appearance of the labeled leucine was slower and more protracted in the casein group, and faster and of shorter duration in the whey group. One explanation of these findings may consist of the casein flocculating in the stomach leading to slower gastric emptying and digestion. This is supported by the finding that ingestion of an amino-acid mixture, reflecting the composition of a specific protein, achieves a much lower whole-body postprandial protein balance than with the intact protein [8]. Nitrogen retention at the whole-body level with ingested whey protein improved, however, when the protein was either administered over time using small boluses or when additional macronutrients (i.e. carbohydrates and fat) were included in the meal [9].

A third observation highlighting the importance of the kinetics of protein absorption is the effect of blood in the digestive tract on the appearance of amino acids in the portal vein. A presumed gastric bleed of 1 liter blood of normal composition delivers an estimated 220 g of protein into the gut (150 g of hemoglobin and 70 g of plasma proteins). This protein is severely deficient in isoleucine because hemoglobin contains no isoleucine and the albumin molecule only 1 molecule of isoleucine. Globulins contain modest amounts of isoleucine. Ingestion of blood in a pig model showed rapid appearance of amino acids in the portal vein, which decreased when isoleucine was simultaneously infused. Urea production also decreased with isoleucine infusion. In humans, a pseudo-bleed (ingesting an amino-acid mixture mimicking the composition of blood) showed diminished peripheral protein synthesis in volunteers [10]. With isoleucine supplementation, appearance of amino nitrogen in the portal vein was slower and more prolonged [10]. This may be explained by improved protein synthesis and temporary retention in the intestine achieved by a more balanced amino-acid mixture. In later years, this phenomenon is called the first-pass effect of a meal. Depending on the tracers used mostly in human studies or on multicatheterized animal models, first-pass effects have been found to be located in the total splanchnic area in human studies and in the intestine and liver separately in animal studies.

A final possibility to improve nitrogen retention is to change the habitual practice in many Western countries of having three meals that typically differ in protein quantity and composition (i.e. the majority of daily protein consumed in the evening meal) over a 12-hour fed period followed by 12 h of fasting. It has been suggested that this practice may render protein retention less efficient than when meals would be of equal size and evenly distributed over the day [11].

In conclusion, in healthy young volunteers and pigs, whole-body nitrogen retention is promoted after a meal only containing protein when appearance of amino acids in the portal vein is tapered. This nitrogen retention may be enhanced by such factors as: (i) including carbohydrate in the meal; (ii) optimizing the amino-acid composition of the protein; (iii) including more slowly digested proteins such as casein as compared to soy or whey; (iv) spreading ingestion of meals over time, and/or (v) ingesting full proteins rather than free amino acids. However, it should be noted that whole-body nitrogen retention of whey as part of a complete meal (i.e. containing carbohydrates) is similar or slightly higher than with a casein-containing meal [12].

Protein Ingestion and Muscle Protein Synthesis in Healthy Volunteers

Despite the improvement in in vivo nitrogen sparing at the whole-body level obtained with isolated casein versus soy or whey protein ingestion (i.e. ingested without other meal constituents), the fractional protein synthesis rate in muscles in young volunteers is highest with whey and lowest with casein [13]. These results do not appear to be in line with the earlier reported improved benefit of casein in comparison with whey in in vivo experiments when measured at the whole-body level. This may be explained by the fact that whole-body protein kinetics, which represent an average response of all proteins in the body, do not necessarily reflect what occurs within muscles. Moreover, the high essential amino-acid content of whey (specifically leucine, which promotes muscle protein synthesis more than any other amino acid) and its rapid absorption rate appears to be an important factor for its greater muscle anabolic effect [13]. However, the interpretation of these findings should be tempered as the technique used did not include assessment of protein degradation and because the limited time frame (i.e. 3 h) precludes the assessment of what could happen in the postprandial period. This is highlighted by a study in which different proteins or amino-acid mixtures were administered and ^{13}C-leucine kinetics were assessed. A rapidly absorbed protein (whey) or an amino-acid mixture led to a greater increase in circulating leucine levels, fractional synthesis rate (FSR) of mixed muscular protein and leucine retention compared with the proteins that are slowly absorbed (casein or whey given in small boluses spread over time) in the first hours [9, 13]. In contrast, whole body net protein balance was more sustained with the slowly absorbed groups, which exhibited after 7 h a greater protein gain than with rapidly absorbed whey or amino-acid mixtures [9]. Ingestion of 6.7 g of essential amino acids containing either 1.7 g (26%) or 2.8 g (41%) leucine in young volunteers stimulated FSR of mixed muscle protein similarly, but only the mix with a higher leucine content increased FSR in the elderly [8]. A supplement containing carbohydrates and amino acids increased phenylalanine uptake to a greater degree within the 2 h after resistance exercise than when carbohydrates or amino acids were administered alone [14]. As outlined earlier, these findings do not convincingly prove that such supplements should be routinely administered to increase muscle mass and fitness in the elderly. However, the combined results of studies with different designs support the view that elderly people maintain better muscle mass and function when protein intake is augmented to 90 g/day (1.2–1.5 g/kg/day) [15, 16].

Protein Ingestion and Muscle Protein Synthesis in the Elderly

The first-pass extraction in the intestine and the total splanchnic area (portal-drained tissues and liver) is well established, and it is generally accepted that more meal-derived amino acids are retained in the splanchnic tissue in 'healthy' elderly than in healthy young volunteers [17, 18]. 'First-pass extraction' may, however, be a misnomer because it is not conceivable that a substantial part of the amino acids derived from protein digestion and absorption, and appearing in the portal vein will be completely and instantaneously taken up in a first pass by the splanchnic tissues and utilized for synthetic purposes. It is more likely that the ratio between the utilization rates of amino acids in central and peripheral tissues shifts to the central tissues operative in host response in the elderly, regardless of whether they entered initially enterally or parenterally. It has been well established that in 'stress' situations there is an amino acid and (indirectly via the liver) glucose flux from peripheral tissues to central tissues and wounded area, serving as substrates for host response, including protein synthesis (collagen, acute-phase, wound and cellular proteins) and synthesis of RNA, DNA and cell membranes (see paper by Soeters [this vol., pp. 17–26]). Simultaneously, inflammatory illnesses lasting longer than a few days and even moderate-sized (surgical) trauma causes an inevitable and visible loss of muscle mass. Therefore, the different utilization of enterally ingested protein in the elderly compared with the younger volunteers supports the view that aging all by itself is an inflammatory condition influencing metabolism possibly even in the rare cases in which truly elderly people have no evident morbidity. It should be pointed out though that inactivity (even something as 'benign' as a reduction in daily step counts for 2 weeks) induces anabolic resistance of muscle protein synthesis, decreases muscle mass and increases markers of chronic low-grade inflammation in otherwise healthy older adults [19]. Therefore, physical (in)activity may have a greater bearing on 'biological' age and its associated negative sequelae than merely the 'chronological' age of an individual. It reflects a continuous low-grade host response in the immune system, including the intestine and liver, leading to rapid turnover of proteins and cell elements in that area. In the elderly, a higher percentage of the meal would therefore be utilized for this purpose than in the younger population, being healthier and less subject to continuous inflammatory stress. This has been suggested to be an important cause for the gradual loss of muscle mass (sarcopenia) occurring on a population basis after the age of 30 years.

The typical generation of cytokines, hormones and other modulators that steer the inflammatory response also contribute to muscle loss, which is therefore partly inevitable. Support for this view comes from efforts to inhibit peripheral protein loss by treating intensive-care patients with growth hormone [20]. Growth hormone had been proven to limit nitrogen losses after trauma and burn injuries [21, 22] but at the same time to increase the number of infectious complications and mortality [20]. It therefore supports the view that peripheral tissues should change their metabolism to furnish substrate for the 'central' immune response and tissue healing (see chapter paper by Soeters [this vol., pp. 17–26]). Apparently, in this process, peripheral tissues become catabolic. Although beneficial in the short term, in the presence of severe long-standing trauma or disease, this arrangement will lead to such a degree of peripheral protein (muscle) loss that patients cannot survive the event. It is therefore important to understand the kinetics of this response and to find ways to limit nitrogen losses without interfering with their benefit. Evidence will be presented that protein intake and exercise can limit whole-body protein loss in aging and possibly in chronic inflammatory disease.

A decade ago, it was found that whole-body leucine balance (a surrogate measure of protein balance) was greater with an isonitrogenous quantity of whey protein compared to casein in both young and older adults when consumed in a mixed meal [12]. These findings at the whole-body level would generally be in line with the greater fractional synthesis of muscle protein in older adults observed with whey as compared to hydrolyzed and micellar casein, which leads to the suggestion that the digestion rate and peak plasma leucine concentration (whey > hydrolyzed casein > micellar casein) is important to consider when targeting muscle protein remodeling with age [23]. In earlier studies, it was found that splanchnic extraction of leucine after a meal in elderly volunteers was approximately double the extraction compared with young volunteers [17]. The percentage of the leucine ingested that was metabolized in the splanchnic tissues decreased with increased plasma leucine levels but the absolute amount retained may have been similar [17]. It was suggested that a higher splanchnic extraction of leucine might lead to lower availability of protein in peripheral (muscle) tissues. This implies that the proportion of the meal utilized in the splanchnic region, wound area and immune system increases with decreasing protein intake, in this way leading to a diminished availability of amino acids for peripheral (largely muscle) tissues. However, it has been suggested on the basis of a subsequent study that the delivery of amino acids to muscle is consistent despite a greater splanchnic extraction [18]. True or not, it is indisputable that aging is generally associated with morbidity and that the immune system, liver, spleen and inflammatory areas exhibit net protein gain whereas peripheral tissues (predominantly muscles) exhibit net protein loss. In view of these contradictory findings, the kinetics leading to the clinical findings earlier mentioned require further investigation.

Notwithstanding the difficulty to obtain a clear picture on the basis of the type of studies referred to, clinical observation learns that any critically ill patient will lose total body protein. This mainly applies to muscle protein, skin and bone, whereas there is clear evidence that simultaneously anastomoses heal, skin defects are epithelialized, immune cells proliferate and the liver accumulates and produces several types of protein operative in host response. Although never studied in a prospective manner, it is likely that intake of meals containing increased amounts of protein (1.5 g/kg body weight/24 h) limits but does not totally inhibit these protein losses in critically ill patients [24–27]. None of these investigations prospectively studied different protein intakes nor their potential effects on muscle protein gain, which arguably weakens the conclusion that increased protein intake is beneficial. If we accept that aging is associated with low-grade inflammatory activity in a similar but less severe manner compared to critical illness, one of the causes of sarcopenia may be chronic inflammation leading to catabolic but probably beneficial muscle metabolism, occurring despite increasing protein intake.

The 'anabolic resistance' of aging mentioned earlier therefore implies that higher doses of amino acids have to be administered to achieve maximal rates of myofibrillar (i.e. contractile) and sarcoplasmic (i.e. noncontractile cellular proteins) protein synthesis, which may [28] or may not [29] reach the same relative levels as in young volunteers. This may be associated with decreased intramuscular expression and/or activation (phosphorylation) of amino-acid-sensing/signaling proteins [29] induced by low-grade inflammation in most elderly people. 'Anabolic resistance' may be a misnomer when after a meal more amino acids are utilized in the splanchnic area leading to a decrease in the amount of amino acids available for muscle protein synthesis and when the same muscle FSR can be reached as in younger people when more amino acids are ingested in the elderly.

Inactivity (in the form of immobilization) also has been associated with 'anabolic resistance' of muscle protein after ingestion of essential amino acids in both young and older adults [30]. However, recent evidence suggests that even a moderate reduction in habitual physical activity is associated with 'anabolic resistance' in skeletal muscle and a marked reduction in lean mass in as little as 2 weeks in otherwise healthy older adults [31]. In contrast, exercise or substantial daily activities can enhance the sensitivity of skeletal muscle to dietary protein. Support for beneficial effects of increasing protein content of the diet in the elderly comes from an observational study in which daily protein intake varied between 54 and 90 g/day [32]. Loss of lean body mass at the 3-year follow-up was (very modestly) diminished in the quintile with the highest protein intake [32]. On the basis of these and other similar studies, it has been concluded that protein requirements in the elderly may be 20–40% higher than in young people, but that additional research employing modern methods has to definitively establish protein requirements [33]. In a very recent epidemiological study, controversy exists regarding the current recommended dietary allowance in young people. A high protein intake was associated with a higher all-cause mortality and mortality from cancer in young people whereas all-cause mortality and mortality from cancer decreased above the age of 65 year [34].

Protein and Amino-Acid Ingestion and Muscle Metabolism in Exercise

Whey hydrolysate has been shown to stimulate the synthesis of mixed muscle protein to a greater degree than soy and casein when given to young individuals in the resting state [13]. Exercise increases the muscle protein synthesis rates after ingestion of these proteins [13, 35]. In healthy elderly men, ingestion of isolated whey protein supports greater rates of muscle protein synthesis than micellar casein both at rest and after resistance exercise [36]. These results are probably related to higher arterial plasma amino-acid or leucine levels with whey ingestion, resulting from rapid absorption and the high content of essential amino acids (specifically leucine) of whey [36]. Rapid aminoacidemia after exercise enhances muscle protein synthesis and the activation (estimated via changes in the phosphorylation status) of anabolic signaling molecules that regulate mRNA translation to a greater extent than an identical amount of whey protein fed in small pulses that mimic a more slowly digested protein in young men [37]. This occurred despite the area under the curve of aminoacidemia being similar after bolus or pulse feeding [37]. It appears that a pronounced peak aminoacidemia after exercise enhances muscle protein synthesis in young and elderly subjects. Essential amino acids stimulate myofibrillar protein synthesis. Leucine has been claimed to have the strongest promoting effects on muscle protein synthesis of all amino acids. In young men, ingestion of 6.25 g of whey supplemented with leucine or a mixture of essential amino acids deficient in leucine stimulated myofibrillar protein synthesis to a similar degree as 25 g of whey in the resting condition, but after exercise this stimulation is only sustained over a longer time period after administration of whey [38]. Mixed muscle protein synthesis at rest and after resistance exercise was similar whether carbohydrates were added or not to a bolus ingestion of whey protein [39]. This is in contrast with another study in which addition of glucose to amino acids augmented the net protein balance (i.e. difference between protein synthesis and protein breakdown) of skeletal muscles 2 and 3 h after resistance exercise [14].

The interpretation of acute measurement of the FSR of muscle after different types of intervention is uncertain because protein degradation is not assessed and the extension of the short duration of the experimental setup to long-term effects may be questioned. However, acute (i.e. ~3 h) measures of muscle protein synthesis and net protein balance across the leg after exercise and nutrition are rather precisely predicting 24-hour response [40], and may qualitatively predict training outcomes such as muscle hypertrophy and lean mass growth [41]. However, long-term training studies provide more insight in the benefit of protein supplementation on muscle mass and strength. Although resistance training unequivocally increases muscle mass and strength independent of age, a recent meta-analysis concluded that protein supplementation can augment these adaptations in both young and older adults [42]. Additional studies are required to fully elucidate the role that protein type and timing (e.g. when it is consumed in relation to an exercise bout) may have on enhancing training outcomes, especially in older adults, as these outcomes may influence adaptive responses in young adults but were not explicitly covered in the meta-analytical approach.

Concluding Remarks

The biological value of protein is dependent on many factors and is arguably related to the specific biological effect and/or tissue of interest. On the basis of the referred literature, whey may be considered to have a high biological value, which is consistent with its high rating on a variety of different scoring systems (e.g. biological value and protein digestibility-corrected amino-acid score). The high absorption rate and content of essential amino acids of whey make it especially suitable for the elderly population who typically present with an 'anabolic resistance' to dietary protein, which may be mediated partly by a greater splanchnic protein turnover that is necessary in host response, and partly by hormones and cytokines steering the inflammatory response. This includes changes in muscle metabolism producing a substrate mix suitable for the synthesis of biomass, immune cells and for the regulation of redox balance (see paper by Soeters [this vol., pp. 17–26]). This effect may also apply to situations of trauma and illness. However, from a standpoint of skeletal muscle, the 'anabolic resistance' of exercise may be mediated by a lack of activity independent of age, which positions physical activity as being of primary importance to improve the sensitivity of muscle to dietary protein. Moreover, exercise or other forms of substantial physical activity are required to promote protein gain and maintain and/or enhance muscle function with dietary protein enhancing these adaptations.

Although the biological value of casein and soy is slightly inferior to whey, these are still good and acceptable proteins that are generally 'made better' (from a muscle standpoint) by prior contractile activity [13]. Aside from the protein source, spreading meals of equal size and composition over the day may optimize protein utilization. A recommended daily allowance of protein of 0.8 g/kg body weight/day has been proposed in the adult population (or an estimated 0.9–1.0 g/kg ideal body weight/day) which recently has been disputed to be an underestimation and that the recommended daily allowance should be 20–30% higher. Athletes, elderly and critically ill individuals may require 1.5–2 g/kg ideal body weight/day. Ultimately, dietary protein is essential for optimal health and well-being given its integral role in lean tissue remodeling and immune surveillance. Therefore, not only the absolute amount but also the quality of protein and the presence of other (macro)nutrients in the meal should be considered when determining the optimal nutrition for a variety of life conditions.

Disclosure Statement

The authors have no conflict of interest to disclose.

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