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Dietary protein requirements and recommendations for healthy older adults – A critical narrative review of the scientific evidence

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Running Title: Protein requirements and recommendations for healthy older adults

List of abbreviations: RDA, Recommended Dietary Allowance; MPS, muscle protein synthesis; MPB, muscle protein breakdown; BMI, body mass index; DXA, dual-energy X-ray absorptiometry; CT, computed tomography; WHO, World Health Organization; FAO, Food and Agriculture Organization; UNU, United Nations University; BV, biological value; PDCAAS, Protein Digestibility Corrected Amino Acid Score; DIAAS, Digestible Indispensable Amino Acid Score.

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Abstract

Adequate protein intake is essential for the maintenance of whole-body protein mass. Different methodological approaches are used to substantiate the evidence for the current protein recommendation, and it is continuously debated whether older adults require more protein to counteract the age-dependent loss of muscle mass, sarcopenia. Thus, the purpose of this critical narrative review is to outline and discuss differences in the approaches and methodologies assessing the protein requirements and hence, resulting in controversies in current protein recommendations for healthy older adults. Through a literature search, this narrative review first summarizes the historical development of the FAO/WHO/UNU setting of protein requirements and recommendations for healthy older adults. Hereafter, we describe the various types of studies (epidemiological studies and protein turnover kinetic measurements) and applied methodological approaches founding the basis and the different recommendations with focus on healthy older adults. Finally, we discuss important factors to be considered in future studies to create evidence for international agreement on protein requirements and recommendations for healthy older adults. We conclude by proposing future directions to determine “true” protein requirement and recommendation for healthy older adults.

Keywords: ageing; sarcopenia; amino acid; protein synthesis; protein breakdown; net protein balance; protein turnover; metabolic adaptation; energy balance; protein intake

1. Introduction

Adequate protein intake is required for the maintenance of whole-body protein mass. The protein mass in a 70 kg adult is about 11 kg. Whole-body protein mass maintenance relies on equal rates of protein synthesis and breakdown, resulting in a zero net balance. The constant kinetics of protein synthesis and breakdown of body proteins were originally demonstrated by Schoenheimer and colleagues in 1939⁽¹⁾ and were termed protein turnover, which is a modifiable and adaptable process. In adult humans, the daily whole-body protein turnover rate is 5.7 g/kg body weight⁽²⁾, meaning that approximately 400 g mixed proteins are turned over every day for a 70 kg adult individual. Behind that number, a large proportion of amino acids are recycled and reutilized for protein synthesis^(3; 4; 5), whereas some are lost via oxidation for energy production and the formation of urea to scavenge nitrogen⁽³⁾. Further, nitrogen-containing substances, such as skin, hair, sweat, urine, and feces are lost from the body. To maintain whole-body protein mass, irreversibly loss of amino acids and nitrogen need to be reconstituted via dietary protein, which founds the basis for the recommended dietary protein intake.

It is well established how much dietary protein should be ingested to account for obligatory nitrogen loss in healthy adults using nitrogen balance methodology^(6; 7). However, it has continuously been debated^(8; 9; 10; 11; 12; 13; 14) whether increasing dietary protein intake in older age is an effective strategy to counteract the age-dependent loss of muscle mass^(15; 16) and strength^(17; 18), a phenomenon termed sarcopenia^(19; 20; 21). However, an increment of the dietary protein recommendation for older adults would categorize numerous people as being protein malnourished and thereby increase the incidence of protein malnutrition worldwide. Such a change would challenge nutritional guidelines and nutritional societies and force governments to act with enormous socio-economic consequences. Thus, it is of utmost importance that we adopt a critical approach to the evidence underlying the dietary protein recommendations.

Independent authorities and expert groups have adopted different criteria and evidence from distinct methodological approaches, resulting in differences in published recommended values of protein recommendation. Consequently, there is currently confusion about the requirement of dietary protein for healthy older adults both in the scientific community and amongst the general older population, who wish to follow the recommendations. Thus, the

purpose of this narrative review is to critically discuss the existing evidence for protein requirements founding the basis for protein recommendations for older adults.

2. Methodology

This narrative review was undertaken by multiple searches: 1) at PubMed and Google Scholar using keywords related to each topic; 2) reference lists were screened for relevant papers; 3) searching at nutritional societies' and authorities' guidelines and references. We specifically included studies conducted in older individuals and only included studies on younger adults when studies in older adults were not available. All articles had undergone peer-review and were available in English.

The first part of this review is a summary of the historical development of the protein recommendation with a brief mention on the approaches used and methodologies applied. In the second part, we present and describe the methodological approaches in more detail that founds the evidence for determining protein requirements and present the key studies providing data for older adults. Finally, we discuss major factors influencing the requirement for dietary protein and thus, needed to be considered in future study designs striving to determine protein requirements. With such consensus, we can accelerate international agreement on protein recommendations for healthy older adults.

3. Historical development of determining protein requirements and recommendations

The first official recommendation for protein intake of 1.0 g/kg/day, published in 1936 by the League of Nations⁽²²⁾, was set based on observations from practice rather than relying on data from a strict scientific approach. Several joint FAO/WHO expert committees have faced challenges in defining “protein requirements”⁽²³⁾. In 1957, FAO/WHO⁽²³⁾ reported their first official protein recommendation and the latest was reported in 2007⁽⁶⁾, which thereafter has been challenged by other expert bodies. **Table 1a** provides an extensive overview of the WHO/FAO/UNU recommendations over the years. While the 2007 recommendation used the definition of protein requirement as “*the lowest level of dietary protein intake that will balance the losses of nitrogen from the body, and thus maintain the body protein mass, in persons at energy balance with modest levels of physical activity...*”, it is uncertain if this amount is sufficient to counteract protein loss in catabolic conditions⁽²⁴⁾. Even though the Expert Committee rigorously reassessed all available data on protein balance in older people, it was concluded that no studies unequivocally demonstrate that the protein requirement

would be higher in older adults when expressed as protein requirement per kg body weight when the purpose was to maintain whole body protein balance.

This has led to the recent challenge of protein recommendation to older adults by some expert bodies. **Table 1b** summarizes major guidelines from external expert bodies, which however are not, official recommendations. Although our understanding of nutritional protein requirement and recommendation have improved over these 60 years, the outcome measurements, and the methodologies on which the outcome measurement is founded have not evolved. The discrepancy in protein recommendations, is partly due to the lack of definitive criterion for “adequate protein”, as well as differences in outcome measurements, which will be discussed in the section 4. Then, important factors that can impact the protein requirement will be reviewed in the section 5.

4. Generation of evidence for protein recommendations for older adults

4.1 Protein/nitrogen balance studies

The nitrogen balance methodology has most widely been used and is considered ‘gold standard methodology’ to establish the protein requirement^(6; 7). Whole-body nitrogen balance is determined by measuring or estimating all nitrogen intake (protein intake) and all excretion and loss (skin, hair, sweat, urine, and feces). A positive balance means excess nitrogen intake relative to losses (i.e., protein accretion within the body). Two crucial prerequisites for a valid measurement are: 1) metabolic adaptation to any given amount of daily protein intake before conducting the measurements^(25; 26; 27), and 2) the achievement of energy balance during the period of measurement⁽⁷⁾. However, the nitrogen balance methodology has its inherent shortcomings: it has been criticized for insufficient sensitivity (i.e. inability to detect a small difference between nitrogen intake and excretion)⁽²⁸⁾. Further, the overestimation of nitrogen intake through food and underestimation of nitrogen loss can lead to erroneous balance measurements.

While there is limited mechanistic insight of the nitrogen balance data^(28; 29), Waterlow and colleagues proposed in 1977⁽³⁰⁾ that a relationship between nitrogen balance and protein balance could be expressed at steady state as: Flux (Q) = protein synthesis (S) + nitrogen oxidations/excretion (O) = protein breakdown (B) + nitrogen input (I) → $Q = I - O = S - B$.

Protein balance calculated as the difference between protein synthesis and breakdown rates provides information about the underlying dynamics of protein kinetics. Protein balance is a rate of either gain or loss of protein per unit time and indicate whether protein mass in the body will quantitatively change over time. Therefore, the prolonged readout of the protein balance data is whole body protein mass and/or changes herein. Whole-body protein mass, often determined by the surrogate measure of muscle mass, is determined in another set of scientific studies, namely the epidemiological studies.

4.2 Epidemiological studies of protein intake

Epidemiological studies, such as cross-sectional and prospective cohort studies can be used for assessing the association between habitual dietary intake patterns and whole-body/muscle mass^(31; 32; 33; 34; 35; 36; 37; 38; 39; 40; 41; 42). Epidemiological studies have the strength that adaptation to a given protein intake level would be inherent, yet they often suffer from several confounding factors. It limits the translation into other settings and makes it impossible to use the results for assessing protein requirements. Longitudinal intervention studies can be applied to examine the cause-effect relationship and/or dose-response relationship between protein intake and most often physiological and functional outcomes, such as muscle mass, muscle strength, and physical function which is particularly relevant to older adults^(43; 44; 45; 46; 47; 48).

4.2.1 Cross-sectional studies

The role of protein intake on the maintenance of muscle mass has been investigated across the lifespan in numerous studies (see **Table 3**). A cross-sectional study from the Framingham Offspring Cohort showed a positive association between the total daily and total animal protein intake and muscle mass after adjustment of physical activity and energy intake⁽⁴²⁾. The association between protein intake and muscle mass became apparent when dividing the cohort into quartiles of protein intake. In men, the difference appeared between the highest (101.1 g/day) and the lowest quartile (64.9 g/day) for total protein intake. In women, muscle mass differed between the highest and the second-lowest quartile for total protein and animal protein intake (93.4 g/day vs. 63.1 g/day)⁽⁴²⁾. Mangano et al.⁽³⁹⁾ examined the third-generation offspring of the original Framingham Heart Study. They divided the cohort into four quartiles based on their protein intake from the lowest intake (quartile 1) 59 g/day (0.8 g/kg/day) to the highest intake (quartile 4) 129 g/day (1.8 g/kg/day). After adjustment for various confounders including physical activity and energy intake, they found a positive association between

protein intake and appendicular lean mass index. A difference was found between the first and the second quartile (80 g/day or 1.1 g/kg/day). However, there was no further difference between the first quartile and the third (99 g/day or 1.3 g/kg/day) and the fourth quartile (129 g/day or 1.8 g/kg/day)⁽³⁹⁾. Collectively, cross-sectional observational studies suggest that protein intake around the international recommendation (0.8 g/kg/day, 60 - 65 g/day in average-sized individuals, 75-80 kg) is sub-optimal for the maintenance of muscle mass throughout adult life. They also suggest that muscle mass may be better maintained when protein intake is higher (80 g/day or 1.1 g/kg/day) than the international recommendation (0.83 g/kg/day), and that no further beneficial effects on muscle mass are observed beyond this amount, suggesting a non-linear association above this level of intake.

Cross-sectional observational studies including healthy older adults have provided an insight into the role of dietary protein intake in the maintenance of muscle mass^(31; 35; 38; 41) (see **Table 3**). A positive association was observed between protein intake and muscle mass^(31; 35). In contrast, it was recently reported that muscle mass did not differ between total daily protein intakes < 0.83 , $\geq 0.83 - < 1.1$, ≥ 1.1 g/kg adjusted body weight in healthy Danish older individuals (≥ 65 yrs.), but this might be due to the small sample size ($n = 25$) in protein intake below 0.83 g/kg/day⁽⁴¹⁾. Geirsdottir et al.⁽³⁵⁾ reported data adjusted for sex, body mass index (BMI), age, and physical activity level and found a higher lean body mass in the quartile with the highest protein intake (1.15 - 1.92 g/kg/day, the fourth quartile) compared to only the first quartile (0.41 - 0.75 g protein/kg/day, $P = 0.04$) and the second quartile (0.76 - 0.92 g/kg/day, $P = 0.05$) but not the third quartile. Interestingly, the differences disappeared when correcting for total energy intake, which emphasizes the crucial importance of considering energy intake when evaluating the impact of protein intake on the regulation of body composition and especially lean body mass in older adults. Similarly, data by Asp et al.⁽³¹⁾ emphasize the dependency between protein and energy intake on muscle and body size as they found that protein intake correlated positively with nutritional status and BMI even when accounting for age, sex, and activity level. In support of protein intake as a determining factor for a lean body composition, Gregorio et al.⁽³⁸⁾ divided a total of 387 older women into those ingesting ≥ 0.8 g/kg/day ($n = 290$) or < 0.8 g/kg/day ($n = 97$) and found that although the women in the ≥ 0.8 g/kg/day group had lower lean mass than those in the < 0.8 g/kg/day group, they also had a lower fat mass, resulting in a lower fat to fat-free ratio in the high-protein group. Collectively, some cross-sectional observational studies have reported positive associations between protein intake and muscle mass in older adults. However, it seems that

when accounting for nutritional status, energy intake and physical activity, there is limited evidence to recommend that protein intake greater than the international recommendation is beneficial for muscle mass preservation.

4.2.2 Observational studies

Association between protein intake and muscle mass was investigated in an observational study design in healthy older adults^(32; 33; 34; 36; 37; 40) (See **Table 4**). A positive association between protein intake and muscle mass was observed in some studies^(32; 33; 34; 36), and these studies concluded that protein intake above the current international recommendation is beneficial in preserving muscle mass in older adults. In all of these studies, baseline average protein intake was higher (~1.2 g/kg/day) than the current international recommendation (0.83 g/kg/day)^(32; 33; 34; 36). In contrast, other studies found that higher protein intake than the current international recommendation does not preserve muscle mass in older adults^(37; 40). Chan et al.⁽³⁷⁾ discussed that the null association between protein intake and muscle mass was due in part to the relatively higher protein intake in this cohort (1.3 and 1.1 g/kg/day in men and women). More recently, Verreijen et al.⁽⁴⁰⁾ reported that protein intake was not associated with the 5-year changes in muscle mass. The contradicting results from Houston et al.⁽³²⁾ were explained by the methodological approaches used to assess muscle mass. Whilst Houston et al.⁽³²⁾ employed dual-energy X-ray absorptiometry (DXA), Verreijen et al.⁽⁴⁰⁾ used computed tomography (CT), which is regarded a more accurate methodology to examine muscle mass. In summary, inconsistent results have been reported in observational studies regarding the association between protein intake above the international recommendation (0.83 g/kg/day)⁽⁶⁾. Thus, the newly suggested protein recommendations for healthy older adults of 0.94-1.3 g/kg/day from different authorities and expert groups^(49; 50; 51) can still be questioned.

4.2.3 Intervention studies

Several randomized controlled intervention studies have investigated whether higher protein intake increases muscle mass in older adults^(43; 44; 45; 46; 47; 48) (see **Table 5**). Some studies were unable to show a beneficial effect of increasing dietary protein intake above their average intake of ~1.2 g/kg/day on muscle accretion or maintenance in older adults^(43; 44; 48). Some studies showing no effect of higher protein intake (≥ 0.8 g/kg/day) tended to have a longer study period (≥ 1 year) compared to studies in which an increase in muscle mass was demonstrated (~ 24 weeks)^(43; 44; 45; 46; 47), suggesting a possible adaptation effect. Mitchell et

al.⁽⁴⁶⁾ showed that protein intake at 2RDA increased muscle mass compared to RDA in a group of men aged >70 yrs. over a period of 10 weeks. However, the individuals in this study consumed a habitual protein intake of 1.1-1.2 g/kg/day on average and the individuals in the RDA group consumed less protein than the habitual intake during the intervention, which may explain the loss of appendicular lean mass and caused a group difference between RDA and 2RDA. For this study, several limitations can be listed, including that energy balance was not maintained in the RDA group; that lack of a steady metabolic state condition as adaptation was not present for the entire intervention period of 10 weeks; and finally, that no habitual protein intake group was included, which therefore does not allow to conclude on changes of lean mass without altered protein intake. In summary, null effects of longer-term intervention of elevated dietary protein intake may indicate that metabolic adaptation may level out acute benefits, which was exemplified by a recent study by Højfeldt et al.⁽⁵²⁾.

4.3 Protein turnover kinetic measurement

Protein turnover kinetic studies are used as more exploratory and mechanistic measurements of the underlying protein turnover kinetic rates. Since the 1970s⁽⁵³⁾, experimental settings with the results of muscle protein synthesis (MPS) based on the stable isotope tracer direct incorporation measurement using precursor-product methods^(54; 55), have established an important basis of knowledge in protein nutrition. In particular, stable isotope tracer and mass spectrometer methodology have advanced our knowledge on MPS in response to amino acids or protein intake^(56; 57; 58). Experiments with infusion of stable isotope amino acid tracers are usually conducted for <24 hours and responses to ingestion of amino acids/proteins or mixed meals are measured in a controlled laboratory setting. Experiments are usually designed to measure postprandial MPS (~ 6 hours) in response to protein intake while participants are kept in artificial conditions such as fasting and bedridden, often with standardized prior dietary intake. The tissue of interest is often skeletal muscle with an emphasis on MPS rate. As muscle protein turnover contributes only approximately 25-35% of whole-body protein turnover in humans^(59; 60), it is important to take this into consideration if the results from these studies are to be used for estimating protein requirement.

4.3.1 Skeletal muscle protein

Amino acid availability in the circulation is a determinant of MPS stimulation⁽⁶¹⁾; a response that is dose-dependent and saturable even in the presence of sustained elevated circulating

amino acids^(62; 63; 64; 65; 66; 67; 68). Although originally hypothesized^(69; 70; 71), recent accumulated evidence has concluded that postabsorptive MPS rates do not differ between older and younger individuals^(72; 73). However, older individuals exhibit a blunted postprandial MPS response to amino acid/protein intake when compared to younger individuals^(63; 74; 75; 76), which has been termed age-related “anabolic resistance”. Moore and colleagues synthesized post-prandial MPS data generated in the lab previously⁽⁶²⁾ and suggested that younger and older individuals are required to consume 0.24 g/kg/meal and 0.4 g/kg/meal, respectively, to maximally stimulate MPS, meaning that approximately 70% greater protein is required to maximally stimulate MPS in older individuals compared to younger individuals.

A blunted MPS, and hence sub-optimal net balance in response to protein feeding and meals could be a plausible driver in the development of sarcopenia. This indicates that older adults need more dietary protein. However, it is important to note that this evidence is derived from acute postprandial MPS studies in response to high quality, rapidly digested, mostly animal-based proteins (e.g. egg, whey, or casein protein)^(62; 76).

In addition to the limitations involved in this experimental design, the potential beneficial effects of higher protein intake for muscle and/or whole-body net protein balance or anabolic response are unknown without the simultaneous measurement of muscle or whole-body protein breakdown. Further, the protein recommendation refers to whole-body protein balance^(6; 7), which is not easily comparable to the responsiveness of skeletal muscle. Hence, we argue that data originating from experimental settings on MPS responsiveness to single dose of protein may lead to misinterpretation and erroneous conclusions on dietary protein requirement in humans at the whole-body level, and therefore cannot be extrapolated to whole-body protein requirement in daily living.

In summary, studies measuring the acute postprandial response to protein feeding with the stable isotope tracer technique have led to the hypothesis that age-related muscle anabolic resistance. It is suggested as one of the underlying mechanisms of the development of sarcopenia^(62; 63; 76). Such quantitative data suggest that protein intake of 0.4 to 0.6 g/kg/meal at 3 main meals and a snack per day is required to preserve muscle mass in healthy older individuals (see **Table 2**), which equates to the protein intake of <1.2-1.8 g/kg/day⁽⁷⁷⁾.

4.3.2 Whole-body protein

More recent investigations have shifted from the use of isolated protein sources to whole foods, which represents the form of protein consumption in everyday life. This shift is due to the interactive effects of protein contained in whole foods with other nutrients and bioactive compounds on muscle and whole-body protein turnover. Studies have primarily focused on MPS in response to beef^(78; 79), eggs^(80; 81), and mixed macronutrient meals^(82; 83). These studies highlighted the importance of considering complex food matrices on the regulation of muscle and whole-body protein turnover.

Previous studies investigating whole-body protein turnover in the context of mixed macronutrient meal intake demonstrated that anabolic response is not limited by protein synthesis^(82; 83; 84). Deutz and Wolfe⁽⁸⁵⁾ advocated that there is no limit to *in vivo* whole-body protein anabolism when protein is consumed as a part of a mixed macronutrient meal^(97; 101). More recently, Park et al.⁽⁸³⁾ showed in healthy older adults (69.3 ± 1.8 yrs.) that there is a higher MPS response following the consumption of a higher protein intake (70 g) compared to moderate (35 g) as part of a mixed macronutrient meal, and a greater whole-body net protein balance in the higher protein intake group due to the suppression of protein breakdown as well as increased protein synthesis (**Figure 1**). Importantly, the doses of protein used in this study (35 or 70 g) were beyond the amount suggested to maximally stimulate MPS in healthy older men⁽⁶²⁾ (see **Table 2**). Similar findings were also found by the same research group in healthy younger adults⁽⁸²⁾.

The suppression of protein breakdown may also be explained by insulin secretion from non-protein energy sources^(86; 87). Higher insulin concentration in circulation is observed when protein is consumed in a mixed macronutrient meal⁽⁸⁸⁾. A blunted protein breakdown suppression⁽⁸⁹⁾ and a failure to achieve positive phenylalanine whole-body net balance^(90; 91) in response to insulin have been reported in older individuals, which may suggest a difficulty in achieving net positive whole-body protein balance in older individuals in response to mixed macronutrient meal intake. A systematic review and meta-analysis concluded that insulin has a permissive role in MPS, whereas insulin attenuates muscle protein breakdown (MPB) independently of amino acids availability^(92; 93). These studies highlight the importance of protein breakdown measurement in the context of a mixed meal intake due to the suppression of protein breakdown in response to higher protein intake and insulin from non-protein energy sources, resulting in increased whole-body protein anabolism. Moving

forward to the justification of the necessity to increase the current international safe level of intake for protein in healthy older adults, the upper limit of protein intake that suppresses protein breakdown, and thus better preserves whole-body protein, should be defined, as highlighted recently by Phillips et al.⁽¹⁴⁾. We argue that whole body protein turnover data and real-life interventions are more appropriate to extrapolate to protein requirements.

5. Future directions of protein requirements and recommendations for healthy older adults

5.1 Energy balance

The importance of energy intake in relation to the determination of protein requirement was raised already by the Joint FAO/WHO ad hoc Expert Committee on Energy and Protein Requirements in 1971⁽⁹⁴⁾, and later in 1981⁽⁹⁵⁾. Notably, Vernon R. Young⁽²⁹⁾ and Joe D. Millward⁽⁹⁶⁾ explored the important concept of protein and energy interrelation to determine protein requirement. The interaction between energy and nitrogen intake on the maintenance of body nitrogen was well documented by studies on the role of non-protein energy sources (carbohydrate and fat)^(97; 98; 99) using nitrogen balance methodology. For example, Collway et al.⁽⁹⁸⁾ showed back in 1954 that exogenous nitrogen-bearing sources contribute to energy production rather than deposition in the body until adequate energy is consumed. Further, increased non-protein energy intake can also mitigate nitrogen loss in a dose-dependent manner⁽⁹⁸⁾. However, the provision of energy attenuates the recruitment of amino acid metabolism for energy turnover and hence retains nitrogen, leading to improvement in nitrogen balance⁽⁹⁸⁾. Accordingly, the achievement of energy balance is an underlying assumption for the current internationally recommended protein intake⁽⁶⁾.

In recent years, protein metabolism and turnover rate have been studied during a condition of negative energy balance^(100; 101; 102) in healthy adults and with an attempt to preserve muscle mass or attenuate muscle loss during negative energy balance by increasing protein intake^(103; 104; 105; 106; 107; 108). The effect of protein intake above the current recommended safe level of intake (0.83 g/kg/day) on muscle mass maintenance during negative energy balance has been well documented^(100; 102; 109). Evidence indicates that whilst amino acids contribute more to energy production at negative energy balance^(105; 110), they are utilized more to de novo protein synthesis when energy balance is achieved. However, evidence is scarce in healthy

older individuals, and it is unclear whether increased protein intake during negative energy balance is sufficient to maintain whole-body and muscle protein mass in healthy older adults.

In contrast, limited knowledge is currently available on the impact of positive energy balance on the regulation of protein turnover rates and muscle mass in healthy older adults.

Nonetheless, based on knowledge from energy deficit studies^(105; 110) as well as studies by Woolfson⁽¹¹¹⁾ and Calloway and Spector⁽⁹⁸⁾, it can be assumed that positive energy balance reduces amino acid oxidation. Accordingly, exogenous amino acids (dietary protein) under such conditions are more efficiently utilized to achieve net positive protein balance. The impact of protein content relative to total energy intake during overfeeding on body composition in younger healthy individuals (18 -35 yrs.) was investigated by Bray et al.⁽¹¹²⁾. Participants were divided into 5% (low protein), 15% (normal protein), or 25% (high protein) of total energy intake from protein after a weight-stabilizing diet period. Following an 8-week intervention, fat mass was similarly increased in all groups because of overfeeding (40% excess energy from a weight-stabilizing diet). However, lean body mass was significantly increased with normal and high protein groups, along with a concomitant increase in resting energy expenditure. These results suggest that energy intake from protein is a sole determinant to increase lean body mass, but not the accretion of fat mass during overfeeding in healthy younger individuals. In this cohort, additional protein may not be required when energy balance is maintained since no impact on lean body mass changes was detected between normal and high protein intake groups⁽¹¹²⁾. Since these findings are limited to healthy younger individuals, further studies are required to investigate in healthy older adults. The concept of interaction between energy balance and protein intake on amino acid oxidation, urea excretion, and whole-body net protein and nitrogen balance at a steady state is illustrated in **Figure 2**.

In summary, energy balance in addition to protein intake is a key determinant of protein turnover rates and net protein balance at the whole-body level. However, energy surplus itself does not seem to increase muscle mass although it reduces amino acid oxidation. Thus, as repeatedly stated in the international reports from WHO/FAO/UNU, energy balance needs to be considered when determining protein requirements. In addition, it is important to highlight that evidence of the interaction between energy balance and protein intake is currently limited to younger individuals, and further evidence is required in healthy older individuals.

5.2 Metabolic adaptation to dietary protein intake

The consideration of metabolic adaptation to any given amount of protein intake is required for a valid measure of protein requirement. Protein turnover in the splanchnic area as well as in the periphery is adaptable to a given amount of protein intake^(25; 26; 27). Metabolic adaptation covers processes affecting the utilization and fate of amino acids, primarily in the splanchnic area. A prolonged exposure to a given amount of protein intake forces enzyme and transporter levels to change accordingly to handle the amino acid availabilities. Hence, metabolic adaptation to a given protein intake level is a fundamental prerequisite when estimating protein requirements. In physiology, *adaptation* covers conditions where achievement of a steady state can be obtained after adjustments of metabolism and physiological function^(113; 114). In contrast, when conditions are too extreme for metabolic pathways to adjust sufficiently, but rather continuously lag behind and the changes are beyond the range of adaptation, the condition is defined as *accommodation*⁽¹¹⁵⁾.

Recent emerging evidence highlight the mechanisms of *adaptation* to a higher protein intake than the current safe level of dietary protein intake. For example, Gorissen et al.⁽¹¹⁶⁾ measured the availability of dietary protein using intrinsically labeled whey protein. In this study, older individuals (62 ± 1 yrs.) were habituated to a protein intake of either 0.7 g/kg/day (LOW) or 1.5 g/kg/day (HIGH) for 2 weeks from a habitual intake of protein at 1.0 g/kg/day.

Interestingly, no group differences were noted in either postabsorptive or postprandial MPS, and more intrinsically labeled whey protein was available in the circulation in LOW (61%) in comparison with HIGH (56%). This was in agreement with our recent study where responses in amino acid and protein metabolism were investigated after habituation to a protein intake of > 2.1 g /kg LBM/day (0.82 g/kg/day) and a protein intake of 1.1 g/kg LBM/day (1.76 g/kg/day) for 20 days in older men (65-70 yrs.)⁽⁵²⁾. These findings suggest that exogenous amino acids are directed less effectively into the circulation when habituated to a high protein intake. Habituation to a high protein intake also resulted in a diminished postprandial synthesis rate of plasma proteins and a more negative overnight fasted whole-body net protein balance, suggesting a less effective utilization of exogenous amino acids for protein synthesis. In accordance, Walrand et al.⁽¹¹⁷⁾ demonstrated that higher protein intake showed a postabsorptive catabolic state, as demonstrated by higher amino acid oxidation and whole-body protein turnover, without changing MPS in both younger (24 ± 1 yrs.) and older individuals (70 ± 2 yrs.) when higher protein intake (3.0 g/kg fat-free mass) was compared to 'usual' protein intake (1.5 g/kg fat-free mass) over 10 days. However, nitrogen balance was

improved in the higher protein intake group, which may suggest insufficient time to adapt to the new protein intake level in this study. Evidence emphasizes the necessity of allowing time for metabolism to *adapt* to increased protein intake. Further evidence adopting study designs and methodological approaches that can account for metabolic adaptation is required to obtain a meaningful value for protein requirement, which can then be translated into recommendations.

Metabolic adaptation to changes in protein intake may explain the null findings when an intervention was performed long enough to achieve adaptation^(26; 52; 118; 119). In other words, sustained increases in muscle mass would not be achieved when individuals chronically consume dietary protein greater than habitual protein intake level. Baseline protein intake level might be a key determinant of the beneficial effect of dietary protein intervention on muscle mass gain in healthy older adults in a randomized controlled intervention study. This notion was highlighted previously⁽¹²⁰⁾ and is supported by a meta-analysis conducted by Ten Haaf et al.⁽¹²¹⁾, where they assessed the impact of protein supplementation on lean body mass, muscle strength, and physical performance in community-dwelling older individuals. Protein supplementation is not beneficial for those outcome measures when sufficient habitual protein intakes are already consumed in non-frail older individuals.

A recent meta-analysis including a total of 8107 community-dwelling older individuals from cohorts in the Netherlands, UK, Canada, and the USA showed that the prevalence of protein intake lower than the currently recommended level of 0.8 g/kg adjusted body weight/day is 14 - 30%⁽¹²²⁾. Thus, this low-habitual protein intake group may be a more relevant target group for protein supplementation intervention with a concomitant focus on ensuring energy requirements. However, when the purpose is to determine whether a higher than currently recommended protein intake is favorable for muscle and whole-body protein mass, healthy older individuals habitually consuming protein around the current safe level of intake should be the target group and *not* the low-habitual intake group. Collectively, more randomized controlled intervention studies are required to conclude whether the newly suggested higher protein intakes from several expert groups above the safe level of intake at 0.83 g/kg/d has a favorable impact on muscle mass in healthy older adults consuming protein at the current international recommendation.

5.3 Protein quality

Protein quality is an overall measure of the ability of a protein source to meet the metabolic demand and is defined in terms of biological value (BV) (i.e., the fraction of amino acids absorbed by the gut from a food that is subsequently retained by the body). BV is expressed in terms of indispensable amino acid patterns relative to the requirement⁽⁶⁾.

The concept of protein quality can be learned from the Ideal Protein Concept developed in the late 1950s by H. H. Mitchell⁽¹²³⁾ and H. M. Scott⁽¹²⁴⁾. The Ideal Protein Concept is defined as the exact amounts of amino acids needed for optimal growth, meaning that it does neither cause amino acid deficiency nor surplus availability. Thus, the Ideal Protein Concept is an effective way to define minimum protein in the diet to meet amino acid requirements for metabolic demands in animals. For example, the Ideal Protein Concept initially attempted to provide diets containing the exact balance of essential amino acids based on the composition of eggs and casein for the maximal growth and production performance of chickens. However, non-essential amino acids were not considered in the concept. Reevaluations in the area of the optimal animal feed for growth have found that the provision of non-essential amino acids is also required for development, growth, survival, reproduction, and health^(125; 126). Consequently, the provision of non-essential amino acids will lower the required amounts of some essential amino acids as they will not be needed as precursors for de novo synthesis of non-essential amino acids. Therefore, protein quality must also be considered when discussing protein requirements and should be reflected in the overall recommendations⁽¹²⁷⁾, including the consideration of non-essential amino acids⁽¹²⁸⁾.

Digestibility of amino acids is an integrated part of the protein quality measure. FAO developed the Protein Digestibility Corrected Amino Acid Score (PDCAAS) as a measure of overall nitrogen digestibility considering loss in fecal matter. This concept had some inherent limitations⁽¹²⁹⁾ that were attempted later to be overcome by replacing it by the new term Digestible Indispensable Amino Acid Score (DIAAS), which includes 1) the adoption of ileal amino acid digestibility, 2) abrogation of truncation of scores, 3) taking the influence of food processing into account, and 4) the use of individual amino acid digestibility.

The earlier protein requirements and recommendations by the WHO/FAO/UNU specified protein quality, whereas the current recommendations refer to 'high-quality protein' with a biological value of 100, usually an animal source protein (see **Table 1a**). Animal-based

proteins are generally accepted as high-quality protein due to better essential amino acid profile and higher digestibility (around 100) as compared to plant-based proteins (ranging 80-85)^(127; 130). Plant-based proteins are less digestible due to existence of dietary fiber and compounds that inhibit enzymatic protein digestion.

Despite the accumulated evidence that supports muscle protein anabolic response by the ingestion of animal-based proteins, plant-based proteins have attracted more attentions due to a sustainable point of view and population health benefits⁽¹³¹⁾. Recently, Burd et al.⁽¹³²⁾ discussed that consideration of protein quality is critical when protein recommendation is determined in relation to an environmental considerations (e.g. managing greenhouse gas emissions to land, water use, and loss of biodiversity). Evidence showed that greenhouse gas emission is lower in plant-based proteins as compared with animal-based proteins⁽¹³¹⁾. Growing evidence has shown that health benefits of vegetarian and vegan diet, including lower incidence and mortality from ischemic heart disease and lower incidence of cancer⁽¹³³⁾. However, the beneficial effects of vegetarian and vegan diet for overall mortality rates are currently unclear⁽¹³⁴⁾.

The impact of vegan and vegetarian diet on muscle mass maintenance in healthy older adults is an important consideration. Cross-sectional studies have showed that total protein and animal protein intake, but not plant protein intake, are positively associated with muscle mass index in older women⁽¹³⁵⁾. Results from a longitudinal observational study show that higher intakes of total protein and animal-based protein are associated with a reduced loss of lean mass over 3 years of follow-up, whereas plant-based protein intake is not associated with lean mass with the fully adjusted models in older adults⁽³²⁾. These data suggest that plant-based diet may not be favourable for muscle mass maintenance in older adults. However, we argue that more studies are required to fully elucidate this association.

Several strategies have been proposed by Gorissen and Witard⁽¹³⁶⁾ to overcome the perceived inferior anabolic properties of plant-based proteins. Firstly, the doses of plant-based protein intake can be increased; secondly, several plant-based protein sources can be mixed in a meal and in the whole diet to overcome any deficiency of a single essential amino acid; thirdly, co-ingestion of leucine can be added as an anabolic stimulant; or fourthly, enhancement of the muscle anabolic sensitivity by physical activity or by other means (e.g. by providing fish oil-derived n-3 polyunsaturated fatty acids in the meal or diet).

Considering potential metabolic roles of individual amino acids is also important. This notion has been exemplified by a study demonstrating that increasing the proportion of leucine to mixed amino acids without altering total amino acids content overcomes anabolic resistance in older individuals⁽¹³⁷⁾. Furthermore, addition of leucine to a suboptimal amount of protein is hypothesized to optimize anabolic response. For example, Wall et al.⁽¹³⁸⁾ demonstrated a greater post-prandial MPS following the consumption of 20 g casein protein with 2.5 g of crystalline leucine compared with no leucine in older men (74±1 yrs.), indicating that modifying and/or supplementing specific amino acid content might be an effective and practical strategy to improve anabolic response. This is particularly relevant to older individuals, where energy requirement and appetite are decreasing with advancing age⁽¹³⁹⁾. Protein requirement that maintains whole-body and muscle protein mass can be achieved with less total protein intake by optimizing protein quality, which is also an important consideration for environmental issues. However, evidence is required whether the modification of protein quality is a feasible approach to maintain whole body protein balance in healthy older adults.

6. Conclusions

Considering the historical development of protein requirements and the use of various research methodologies to create scientific evidence for assessing protein requirements to found recommendations, we found that the previously used whole-body nitrogen balance methodology is challenged by methods evaluating whole body and/or muscle mass and whole body and/or muscle protein turnover rate as criteria. The newly suggested protein recommendations (0.94 - 1.3 g/kg/day) formulated by some authorities and expert groups target muscle mass maintenance instead of whole-body protein mass as their primary outcome criterion.

To close the knowledge gap between protein requirements for maintaining both muscle mass and whole-body mass, we identified that future research should assess the degree of agreement between those different though related outcome measures based on distinct methodologies. We claim a strong need for prospective longitudinal studies with frequent monitoring of reliable dietary intake and concurrent measurements of whole-body protein mass and muscle mass with multiple methodologies. These would include short-term measurements (e.g., muscle and whole-body protein balance, kinetic rates, and nitrogen fluxes) and longer-term measurements (ensuring metabolic adaptation and energy balance) with consideration of protein quality. Preferably also evaluating clinically relevant outcomes, such as muscle strength and function, physical function, body composition, and metabolic health parameters would be relevant information. Such a holistic experimental approach would support establishing an agreement between muscle and whole-body protein mass maintenance, and thereby reveal the “true” and healthy dietary protein requirements and recommendations for healthy older adults.

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Authorship

Yusuke Nishimura: Conceptualization, Visualization, Writing - original draft, Writing - review & editing. **Grith Højfeldt:** Writing - review & editing. **Leigh Breen:** Writing - review & editing. **Inge Tetens:** Conceptualization, Writing - review & editing. **Lars Holm:** Conceptualization, Writing - review & editing. All authors read and approved the final manuscript.

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Table 1a. Successive protein requirements and recommendations by international groups to ensure nitrogen balance in adults

Report	Age	Methodological approach	Biological value of dietary protein (%)	Average protein requirements (g/kg/day)	Recommendation/ safe level of intake (g/kg/day)
League of Nations 1936 ⁽²²⁾	Adults	-	-	-	1.0
FAO 1957 ⁽²³⁾	Adults	N-balance	80	0.53	0.66
FAO/WHO 1965 ⁽¹⁴⁰⁾	Adults	Factorial	80	0.71	0.89
FAO/WHO 1973 ⁽⁹⁴⁾	Adults (20-39 yrs.)	Factorial	75	0.57	0.75
FAO/WHO/UNU 1985 ⁽⁹⁵⁾	Adults	N-balance	100	0.75	0.75
FAO/WHO/UNU 2007 ⁽⁶⁾	Adults (\geq 18 yrs.)	Meta-analysis (N- balance studies) ⁽⁷⁾	100	0.66	0.83

Adopted from NS Scrimshaw⁽¹⁴¹⁾ and updated.

Table 1b. Protein recommendations by expert bodies to maintain muscle mass and strength in older adults

Report	Age	Methodological Approach/study design	Functional outcomes	Recommendation (g/kg/day)	Rationale
Nutrient Reference Values for Australia and New Zealand 2006 ⁽¹⁴²⁾	> 70 yrs.	Factorial	Estimation of the amount needed for growth and maintenance on a fat-free mass basis	Men, 1.07 (81 g/day) Women, 0.94 (57 g/day)	Based on Rand et al. ⁽⁷⁾ and additional studies ^(7; 143; 144) , the average requirement was increased by 25% over that of younger adults.
The Nordic Nutrition Recommendations 2012 ⁽⁵¹⁾	> 65 yrs.	Systematic review ⁽¹⁴⁵⁾ (prospective cohort, case control, and intervention studies)	N-balance Muscle mass Muscle strength Bone mass Morbidity Mortality	1.1-1.3 (15-20 E% and the protein E% should be increased with decreasing energy intake)	The grade of evidence was assessed as <i>convincing</i> , <i>probable</i> , <i>suggestive</i> , and <i>inconclusive</i> ⁽¹⁴⁵⁾ . The evidence of protein requirement (0.66 g/kg/day) and recommendation (0.83 g/kg/day) based on N-balance studies was assessed as <i>probable</i> . The evidence from prospective cohort studies in relation to functional outcomes (e.g., muscle mass and muscle strength) with a safe intake of at least 1.2-1.5 g/kg/day was assessed as suggestive to inconclusive.
The PROT-AGE Study Group 2013 ⁽⁴⁹⁾	> 65 yrs.	Epidemiological studies Clinical trials	Muscle mass Muscle strength Physical function	1.0-1.2	Decrease in anabolic response to protein intake in older adults; higher protein intake is required to offset

			Muscle protein synthesis		inflammatory and catabolic conditions often observed in older adults. Both endurance and resistance exercise were recommended, and even higher protein intake (> 1.2 g/kg/day) was recommended those who engage in exercise.
The ESPEN Expert Group 2014 ⁽⁵⁰⁾	> 65 yrs.	Epidemiological studies Clinical trials	Muscle mass Muscle strength Physical function Muscle protein synthesis	1.0-1.2	Older adults require higher protein due to anabolic resistance, low postprandial amino acid availability, decreased muscle perfusion, sarcopenia, and disease-related protein catabolism. Both endurance and resistance exercise were recommended.

Table 2. Protein recommendation derived from acute stable isotope tracer incorporation studies in younger and older adults

	Younger	Older
Dietary protein	(g/kg BM)	
Single meal	0.24	0.40
Day (3-4 meals)	0.72 - 0.96	1.2 - 1.6
Dietary protein	(g/kg LBM)	
Single meal	0.25	0.61
Day (3-4 meals)	0.75 - 1.0	1.83 - 2.44
Dietary protein	(g) ¹	
Single meal	18	30
Day (3-4 meals)	54 - 72	90 - 120

¹An individual with 75 kg of body mass (BM).

LBM, lean body mass.

Values were adopted from Moore et al.⁽⁶²⁾ determined by Biphasic Linear Regression Model from 6 independent studies^(64; 65; 146; 147; 148; 149).

Table 3. Selected cross-sectional observational studies assessing the association between protein intake and muscle mass

Author, location	Subjects Men (M), Women (W)	Age, years	Dietary assessment	Body composition measurement	Average protein intake	Protein intake	Outcomes	Additional information
Sahni et al. 2015 ⁽⁴²⁾ , USA	1139 M, 1497 W	29- 86	FFQ	DXA	M: 80 g/day W: 76 g/day	g/day Q1: M 64.9, W 57.8 Q2: M 70.8, W 63.1 Q3: M 79.2, W 73.5 Q4: M 101.1, W 93.4	A positive association between protein intake and leg lean mass	
Mangano et al. 2017 ⁽³⁹⁾ , USA	2905 M&W	19- 72	FFQ	DXA	93 g/day	g/day (g/kg/day) Q1: 59 (0.8) Q2: 80 (1.1) Q3: 99 (1.3) Q4: 129 (1.8)	A positive association between protein intake and ALM	

Geirsdottir et al. 2013 ⁽³⁵⁾ , Iceland	99 M, 138 W	65-92	3-d food record	DXA	M: 90.3 g/day, 19.6 E% W: 69.6 g/day, 18.8 E%	g/kg/day Q1: 0.63 Q2: 0.85 Q3: 1.01 Q4: 1.36	A positive association between protein intake and LM. Higher LM in Q4 vs Q1 (2.3 kg LM difference)	Significance was not evident after the correction with energy intake
Asp et al. 2012 ⁽³¹⁾ , USA	47 M, 95 W	60-88	DHQ	CC	15.5 E%	Protein E%	A positive association between protein intake and CC	A positive association between beef protein intake and CC
Gregorio et al. 2014 ⁽³⁸⁾ , USA	387 W	60-90	4-d food record	DXA	72.2 g/day, 1.1 g/kg/day	g/kg/day < 0.8 ≥ 0.8	LM was lower in ≥ 0.8 compared to < 0.8. Fat-to-Lean Ratio was lower in ≥ 0.8 g/kg/day	
Højfeldt et al. 2020 ⁽⁴¹⁾ , Denmark	98 M, 86 W	65-82	3-d food record	DXA	82.8 g/day, 1.13 g/kg/day, 17.6 E%	g/kg aBW/day < 0.83 ≥ 0.83 - < 1.1 ≥ 1.1	ASMI did not differ between different protein intakes	

FFQ, food-frequency questionnaire; E, energy; DXA, Dual-energy X-ray absorptiometry; Q, quartile; DHQ, diet history questionnaire; CC, calf circumference; aBW, adjusted body weight; LM, lean body mass; ALM, appendicular lean body mass; ASMI, appendicular skeletal muscle index.

Table 4. Selected observational studies assessing the association between protein intake and muscle mass

Author, location	Subjects Men (M), Women (W)	Age, year s	Dietary assessm ent	Body composit ion measure ment	Follow- up duratio n	Baseline average protein intake	Baseline protein intake	Energy intake/balance	Outcomes
Houston et al. 2008 ⁽³²⁾ , USA	967 M, 1099 W	70- 79	FFQ	DXA	3 years		% of energy (g/kg/day) Q1: 11.2 (0.7) Q2: 12.7 (0.7) Q3: 14.1 (0.8) Q4: 15.8 (0.9) Q5: 18.2 (1.1)		Higher protein intake was associated with higher preservation of LM and aLM
Meng et al. 2009 ⁽³³⁾ , Australia	862 W	70- 85	FFQ	DXA	5 years	81 g/day, 1.2 g/kg/day, 19 E%	g/day, g/kg/day (E%) Q1: <66, 0.84 (17.7) Q2: 66-87, 1.17 (19.0) Q3: >87, 1.64 (20.4)		Higher baseline protein intake was associated with higher LM and aLM at the 5-y follow-up

Scott et al. 2010 ⁽³⁴⁾ , Australia	370 M, 370 W	50-79	FFQ	DXA	1.4-4.8 years (mean 2.6 years)	87.6/day, 1.13 g/kg/day	< RDI ≥ RDI	Energy intake and BW were maintained between baseline and follow-up. Step counts were decreased in follow-up compared to baseline.	Higher protein intake was associated with aLM at baseline and the follow-up
McDonald et al. 2016 ⁽³⁶⁾ , Denmark	39 M, 40 W	>65	Interview	Bioelectrical impedance	6 years	74.6g/day, 1.07 g/kg/day	g/kg/day Q1: 0.61 Q2: 0.92 Q3: 0.97 Q4: 1.26		Higher protein intake was associated with higher preservation of LM
Chan et al. 2014 ⁽³⁷⁾ , China	1411 M, 1315W	≥65	FFQ	DXA	4 years	1.3 and 1.1 g/kg/day for M and W	g/kg/day Q1: ≤0.9 Q2: 0.91-1.2 Q3: 1.1-1.6 Q4: ≥1.61		No association was found between protein intake and ASM

Verreijen et al. 2019 ⁽⁴⁰⁾ , USA	749 M, 812 W	70- 79	FFQ	CT	5 years	66.0g/day, 0.90 g/kg/day	g/kg/day Q1: 0.50 Q2: 0.68 Q3: 0.85 Q4: 1.03 Q5: 1.39	Protein intake was not associated with 5-year change in muscle cross- sectional area
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FFQ, food-frequency questionnaire; E, energy; DXA, Dual-energy X-ray absorptiometry; Q, quartile; CT, computed tomography; RDI, recommended dietary intake; BW, body weight; LM, lean body mass; ASM, appendicular skeletal muscle mass; aLM, appendicular lean body mass.

Table 5. Selected randomized controlled trials investigating the impact of protein intake on muscle mass in healthy older adults

Author, location	Study design	Subjects		Follow-up duration	Dietary assessment	Body composition measurement	Baseline average protein intake	Energy intake/balance	Intervention	Outcomes
		Men (M), Women (W)	Age, years							
Mitchell et al. 2017 ⁽⁴⁶⁾ , New Zealand	Parallel	29 M	70-81	10 weeks	3-d food record	DXA	88-101 g/day, ~1.2 g/kg/day, 14-17 E%	Energy intake was decreased in post from pre in RDA (-440 kcal) and increased in 2RDA (+555 kcal). BW was decreased in RDA (-2.1 kg) and 2RDA (-0.5 kg).	Protein intake at RDA (0.8 g/kg/day) vs 2RDA (1.6 g/kg/day)	2RDA increased LM (+1.49 kg) compared with RDA (-0.55 kg)
Ten Haaf et al. 2019 ⁽⁴⁷⁾ , Netherlands	Parallel	93 M, 21 W	67-73	12 weeks	Two 24h recalls	DXA	0.89 g/kg/day, 16 E%	Energy intake was reduced in post from pre	A total of 31 g of protein in 500 ml milk	Protein supplementation increased LM

								in both protein (-78 kcal) and placebo (-10 kcal) groups. BW was decreased in protein (-0.59 kg) and placebo (-0.15 kg) groups.	protein concentrate drink per day or placebo	(+0.93 %) compared to placebo (+0.44%)
Norton et al. 2016 ⁽⁴⁵⁾ , Ireland	Parallel	14 M, 46 W	50-70	24 weeks	4-d food record	DXA	83-86 g/day, 1.2 g/kg/day, 16-19 E%	Energy intake was increased in post from pre in protein (+111 kcal) and placebo (+116 kcal) groups.	A total of 145.4 g of protein per day or placebo	Protein supplementation increased LM (+0.45 kg) compared to placebo (-0.16 kg)
Mertz et al. 2021 ⁽⁴⁸⁾ , Denmark	Parallel	184 M&W	65-82	1 year	3-d food record	MRI/DXA	82.8 g/day, 1.1	Energy intake was increased in post from	A total of 1) 40 g whey protein, 2) 40 g	Protein supplementation did not increase

								g/kg/day, 17.6 E%	pre in whey (+518 kcal), collagen (+408 kcal), and placebo (+949kcal). BW was increased in whey (+0.4 kg), collagen (+0.7 kg), and placebo (+1.2 kg).	collagen protein, or 3) placebo	LM and CSA
Kerstetter et al. 2015 ⁽⁴³⁾ , USA	Parallel	30 M, 178 W	75 (mean)	18 months	3-d food record	DXA	72.9 g/day, ~1.07 g/kg/day	Energy intake was decreased in post from pre in protein (-120 kcal) and placebo (-4 kcal) groups. BW was	A total of 45 g whey protein or placebo	Protein supplementation did not increase LM	

maintained in
both groups.

Zhu et al. 2015 ⁽⁴⁴⁾ , Australia	Parallel	181 W	70-80	2 years	3-d food record	DXA	76 g/day, 1.1-1.2 g/kg/day	BW was increased in protein (+0.5 kg) and placebo (+0.4 kg).	A total of 30 g of skim milk- based protein supplement or placebo	Protein supplementation did not increase LM
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DXA, Dual-energy X-ray absorptiometry; E, energy; BW, body weight; LM, lean body mass; RDA, recommended dietary allowance; MRI, magnetic resonance imaging; BW, body weight; CSA, cross sectional area.

Figure legends

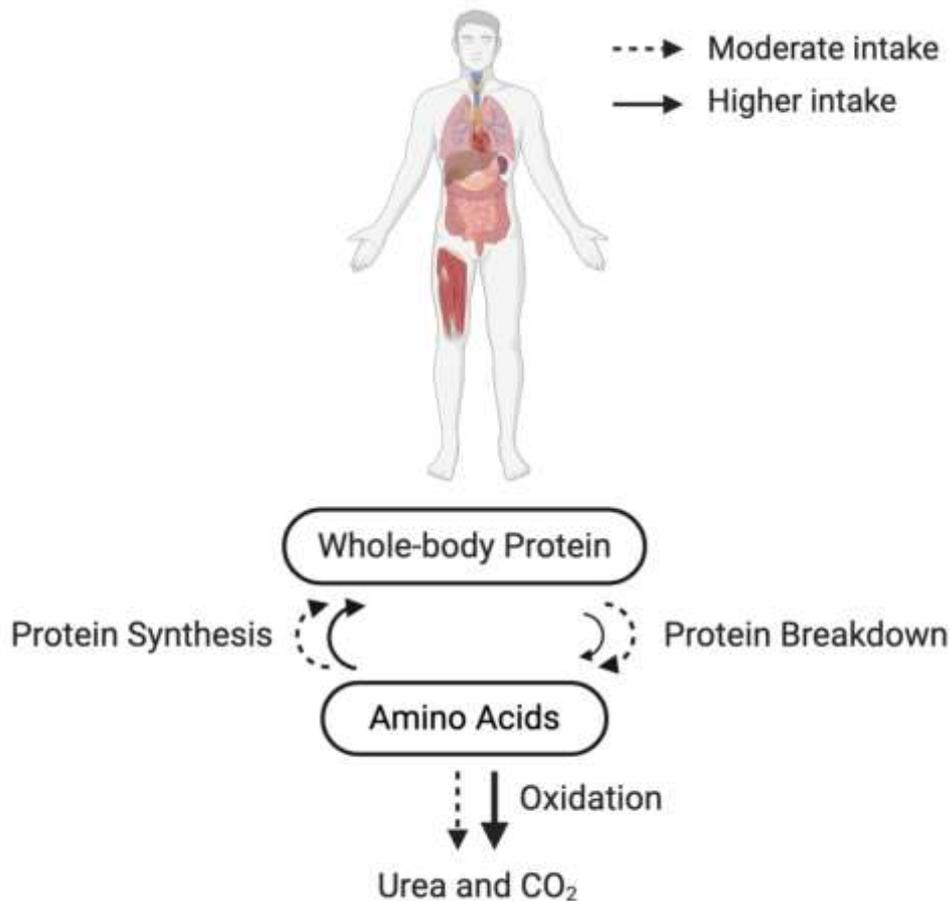


Figure 1: Whole-body protein turnover in response to the recommended or higher protein intake in a mixed macronutrient meal. Dashed (- -) and solid (—) lines indicate a moderate (~35 g/meal) and a higher protein intake (~70 g/meal) in a mixed macronutrient meal, respectively. Protein synthesis is saturable at the given amounts of protein intake⁽⁸²⁾ – illustrated by the similar-sized arrows for protein synthesis between the moderate and higher protein intake. Protein breakdown is suppressed by the higher protein intake in a mixed macronutrient meal (illustrated by a smaller solid arrow), resulting in a greater whole-body net protein balance^(82; 83; 85; 150). Be aware that a higher protein intake (i.e. surplus of amino acids) also inevitably increases amino acid oxidation and urea excretion^(52; 117). The results depicted in this figure were originated from collective data based on stable isotope tracer studies.

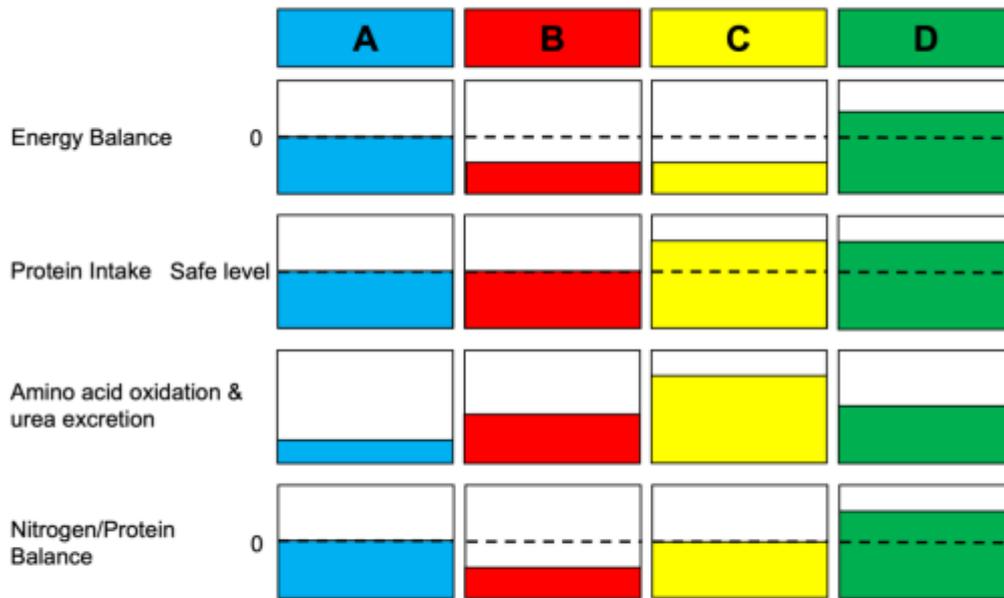


Figure 2: Interaction between energy and protein intake on amino acid oxidation, urea excretion, and whole-body net nitrogen and protein balance during the condition of adaptation. In each column, relative energy balance, protein intake, amino acid oxidation & urea excretion, nitrogen/protein balance are expressed. For energy balance and nitrogen/protein balance, 0 (dashed line) indicates that a balance is maintained. Safe intake (dashed line) in protein intake shows a protein intake recommended by WHO/FAO/UNU (0.83 g/kg/day). **(A)** the column indicates zero whole-body net nitrogen and protein balance at the safe level of intake for protein recommended by WHO/FAO/UNU under energy balance condition⁽⁶⁾. **(B)** and **(C)** indicate a negative energy balance condition. **(B)** the column shows the protein intake at the safe level of intake, but amino acid oxidation and urea excretion are increased under a negative energy balance condition, leading to negative whole-body net protein and nitrogen balance during negative energy balance^(105; 110). **(C)** the column demonstrates that an increased protein intake (> 0.83g/kg/day) preserves whole-body net protein and nitrogen balance whilst increasing amino acid oxidation and urea excretion under a negative energy balance condition^(104; 107; 108). **(D)** the column denotes that amino acid oxidation and urea excretion are reduced under a positive energy balance condition with an increased protein intake (> 0.83g/kg/day), resulting in positive whole-body net protein and nitrogen balance⁽¹¹²⁾.