Aging, protein requirements, and protein turnover1–3

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ABSTRACT  Current protein requirements for the elderly derive from 1985 FAO/WHO/UNU recommendations of no change with age in adults: i.e., 0.6 g/kg average and 0.75 g/kg safe allowance. Although concern has been expressed that protein requirements for the elderly may be greater, a review of nitrogen balance data, none of which are entirely satisfactory, indicates little reason for any revision. Furthermore, the 1985 recommendation is generally consistent with reports that the rate of whole-body protein turnover, a commonly assumed determinant of the protein requirement, exhibits minimal change with age per unit fat-free mass. Recent novel tracer studies aimed at evaluating protein requirements and turnover in a systematic way also support the 1985 recommendations. [1-13C]leucine balance studies have allowed measurement of metabolic demand from postabsorptive leucine oxidation and the efficiency of protein utilization from changes in leucine balance with feeding. The apparent protein requirement is metabolic demand divided by efficiency, an indication of protein needs and utilization during a standardized protocol at intakes similar to habitual ones. In healthy, mobile, elderly persons, metabolic demands are reduced by about one-third, with no significant impairment in efficiency of protein utilization. Thus, apparent protein requirements appear to fall with age from 0.98 ± 0.17 to 0.69 ± 0.22 g/kg. These changes with age reflect an improved restraint of proteolysis in the postabsorptive state, with little change with age in whole-body protein synthesis. The requirements of frail and immobile elderly and the efficiency of protein utilization of meals as eaten by elderly people remain to be evaluated. Am J Clin Nutr 1997;66:774–86.

KEY WORDS  Protein requirement, protein turnover, aging, leucine oxidation, stable isotopes

INTRODUCTION

The nature of the problem

Defining human protein requirements has historically been difficult and consequently controversial. As with all nutrients, any consideration of the issue requires answers to questions of both how much? and what for? For protein, the difficulty of addressing the second question prevents an entirely satisfactory answer to the first. In the case of "how much?" the 1985 FAO/WHO/UNU report (1) that forms the basis of current requirements argues that healthy elderly people have a dietary protein requirement that is not less than the need established from nitrogen balance studies in younger adults, i.e., an average requirement of 0.6 g protein/kg, with a safe protein intake of 0.75 g/kg, to reflect individual variability in requirements. It was argued in the report (1) that "This figure is higher than that for younger adults in relation to lean body mass" (recognizing the reduced amount of lean tissue in this group), "because it is an accepted fact that protein utilization is less efficient in the elderly." The basis of this "accepted fact" is unclear because little is known about the extent and regulation of protein utilization in humans. However, these conclusions have been adopted by all subsequent national committees (2, 3).

In these various reports (1–3), the issue of "what for?" has been discussed only to a limited extent, i.e., in the context of the need to maintain protein turnover, with adaptive reductions in response to low intakes to avoid deficiency (recognized as rare) and limited to responses of children (eg, stunting, poor muscle, and kwashiorkor-like pathology) (2). Although these are commonly held views, it is by no means certain that any of them are correct. As far as responses of protein turnover to protein deficiency, the most recent comprehensive studies indicate that there may be little influence of variation in intake, even at low intakes on the overall replacement rate (4). There is also both uncertainty and active debate about whether any of the usually associated deficiency symptoms in children do reflect protein deficiency as opposed to infection, micronutrient deficiencies, or both (5). In the 1991 UK COMA report of dietary reference values (3) the issue was not even discussed, with protein requirements defined only in terms of "how much" (the rate of weight gain or growth and the achievement of a suitably positive nitrogen balance); the discussion of "what for" was limited to "maintenance of a state of well-being."

One focus of concern in relation to protein nutrition in the elderly, which could conceivably form the basis of "what for?" is sarcopenia—the selective loss of skeletal muscle mass. This is a major component of the age-related decline in muscle strength. The extent of this is variable between individuals, between the sexes, and within individuals according to muscle group, but includes a reduction in dynamic, isokinetic, and static strength (6). Cross-sectional and longitudinal body-composition studies (7–9) together with autopsy dissection data

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indicate an overall decline in skeletal muscle mass from 45% in young adults, to < 27% after the age of 70 y, with little change in nonmuscle lean tissue.

Apart from the serious functional consequences, the metabolic consequences of sarcopenia are usually discussed in terms of muscle as a major reserve of readily available amino acids in times of stress. Thus, sarcopenia could diminish the capacity of elderly individuals to respond successfully to any stress requiring amino acids, such as the hepatic acute phase response or support of the immune response. The best understood component of muscle’s functional reserve capacity relates to its normally very large glutamine pool. Glutamine can be selectively released because of its highly regulated specific transport system (10, 11), enabling its multifunctional metabolic and immune function role to be achieved. In mouse neutrophils function is compromised during both aging and protein deficiency (12), and because in critically ill patients the extent of muscle wasting is a major determinant of outcome (13, 14)—with glutamine supplementation improving survival in intensive care patients (15)—there is every reason to be concerned about sarcopenia in the elderly.

The main determinant of sarcopenia appears to be the decline in resistance-type physical activities (16–20). Nevertheless, it must be assumed that an adequate dietary protein intake is also a prerequisite for healthy aging and it is clearly important to identify the extent of any nutritional influence on this decline in relation to both muscle strength and the metabolic implications of sarcopenia. Because such information is not currently available, it is only possible to address limited but nevertheless very important questions relating to the protein requirement of the elderly. These questions are as follows: 1) Do the conclusions reached by the 1985 report (1) remain valid today? and 2) Do measurements of age-related changes in protein and amino acid turnover afford any additional insight into the nutritional needs of the elderly? An examination of nitrogen balance data and isotopic studies of nitrogen and amino acid turnover and oxidation are required to answer these questions.

**NITROGEN BALANCE STUDIES OF PROTEIN REQUIREMENTS IN THE ELDERLY**

Information available to the 1985 Expert Consultation (1) on the effects of age on protein requirements to maintain nitrogen balance was limited and conflicting. It had been shown that young and elderly subjects had similar responses to a low-protein diet in terms of the decreases in urinary nitrogen losses (21) and the extent of obligatory nitrogen losses per kilogram body weight (22), but few multipoint balance studies had been published. Furthermore, whereas relatively little additional data has been reported since the 1985 report, a recent reassessment of the published data in the context of a new nitrogen balance study (23, 24) concluded that the protein requirements of the elderly should be increased to 1–1.25g · kg⁻¹ · d⁻¹. However, the interpretation of nitrogen balance data is especially difficult in the case of the elderly, not least because there are more constraints on experimental design than with studies of younger subjects. Consequently, any revision of the 1985 recommendations should only be made on the basis of a thorough evaluation of all pertinent information.

Published nitrogen balance studies on the elderly were reviewed recently (25), and the results of some are given in Table 1. In reviewing this data, Millward and Roberts (25) identified several important problems. Propagation of errors, both random and systematic, reduced both the accuracy and precision of the nitrogen balance measurements. In addition, because interactions between energy and nitrogen balance raise both practical and theoretical problems, nitrogen balance studies must be conducted in individuals in energy balance. However, because energy requirements for the elderly are higher

### Table 1

<table>
<thead>
<tr>
<th>Study and reference</th>
<th>Subjects</th>
<th>Intakes</th>
<th>Authors’ conclusions</th>
<th>Current reassessment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Examination of any influence of age on the protein requirement</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1, (27) 8M</td>
<td>7M</td>
<td>0.4, 0.8, 1.6</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>2, (29, 30)</td>
<td>19M, 10F</td>
<td>1.0, 1.8</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>17M, 11F</td>
<td>0.9, 1.5</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Measurement of requirement intake in older subject</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3, (28)</td>
<td>7M</td>
<td>0.57, 0.70, 0.85</td>
<td>—</td>
<td>0.7–0.85</td>
</tr>
<tr>
<td></td>
<td>7F</td>
<td>0.52, 0.65, 0.8</td>
<td>—</td>
<td>0.83</td>
</tr>
<tr>
<td>4, (26)</td>
<td>6M</td>
<td>0.04, 0.38, 0.44</td>
<td>—</td>
<td>0.46</td>
</tr>
<tr>
<td></td>
<td>12M + F</td>
<td>0.8, 1.62</td>
<td>—</td>
<td>1.0</td>
</tr>
<tr>
<td>5, (23)</td>
<td>12F</td>
<td>0.45, 0.92</td>
<td>—</td>
<td>0.78–0.82</td>
</tr>
<tr>
<td>6, (31)</td>
<td>8M</td>
<td>0.8</td>
<td>—</td>
<td>&gt; 0.8</td>
</tr>
<tr>
<td>Measurement of adequacy of single intake</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7, (32)</td>
<td>7M, 8F</td>
<td>0.8</td>
<td>—</td>
<td>&lt; 0.8⁶</td>
</tr>
</tbody>
</table>

¹ Adapted from reference 25.
² Energy intakes were low.
³ Subjects depleted during balance periods.
⁴ Group in overall balance at lowest intake.
⁵ No intermediate intakes, prevents calculation of actual requirement.
⁶ Group achieved overall balance with the chosen intake.
than previously believed (33), and because several documented studies of nitrogen balance in the elderly were conducted at quite low energy intakes, existing nitrogen balance data for the elderly may overestimate the protein requirement. Indeed, with energy and protein balance being interdependent it is by no means a simple task to identify whether subjects failing to maintain nitrogen balance do so because of a deficit of protein as opposed to a deficit of energy intake. Miscellaneous nitrogen losses are seldom measured but usually assumed to be 8 mg N/kg, so that evidence of lower values for the elderly can lead to an underestimation of nitrogen balance and overestimation of protein requirement. Some studies are based on shorter periods than the minimum 5 d deemed necessary to allow for day-to-day variability in nitrogen balance. Indeed, the time required for adaptation raises difficult problems because with protein intakes typically 1.5–2.0 times higher than currently recommended average needs, it is seldom possible to allow sufficient time for complete adaptation to the low protein amounts fed in balance trials. In fact, one of the explanations for the variation in outcome of balance studies is undoubtedly the different approaches to adaptation taken in published studies. In summarizing the expected bias in nitrogen balance studies, Millward and Roberts (25) concluded that the combined effect would be a tendency toward a false overestimation of protein requirements.

There is a wide range of designs and analytic approaches to nitrogen balance measurements that can markedly influence results. With the nonlinearity of the nitrogen balance–nitrogen intake curve as it approaches equilibrium, linear regression of a selected part of the balance curve results in varying zero-balance intercepts according to which intake values are chosen. Published studies include analysis of both submaintenance intakes, which predict a low requirement, and of mainly high intakes, which predict a higher requirement. Recent reports that include both new data (23, 24) and reanalysis of previous data (26–28) exemplify these difficulties. Although the authors concluded that their reanalysis shows an increased requirement in the elderly, the way these conclusions are reached makes them by no means certain (25).

In fact, Millward and Roberts (25) concluded that none of the studies in Table 1 are entirely satisfactory and certainly none of the studies conform to the protocol identified by UNU/WHP (34) as appropriate for the establishment of the protein requirement by nitrogen balance. All the studies differ considerably in terms of their experimental design and to some extent in their stated objectives. Only two studies, study 1 (27) and study 2 (29, 30) specifically addressed the question of the extent of any age-related changes in protein requirements by studying both younger and older subjects. No differences were identified and the design did not allow a requirement value to be predicted with any confidence, even after correction for miscellaneous nitrogen losses. None of the four studies with the stated aim of identifying a protein requirement for the elderly were ideal. Thus, in study 3 (28), low energy intakes (1.33 × basal metabolic rate) and generally high protein intakes were used, which probably accounts for the higher mean requirement (0.8 g/kg) compared with the requirement derived for younger adults published by the same laboratory. The design of study 4 is problematic given the chosen intakes (very low and two similar low intakes); therefore the regression of this data will underestimate requirements (26). The conclusions of both study 5 (23) and study 6 (31) of a mean requirement between 0.78 and 1.0 g/kg can be viewed as predictable from the intakes chosen for the studies and as indicative of a requirement probably <0.8 g/kg but not identifiable without further studies at intermediate intakes. The final study in Table 1, study 7, was a 30-d balance study aimed to test the adequacy of an intake of 0.8 g/kg. The results were interpreted as indicating a mean protein requirement >0.8 g/kg, but involved energy intakes that may well have been inadequate, and nitrogen balances for the group as a whole that did not differ significantly from zero balance (32). Thus, none of these published studies provide convincing evidence that the protein requirement for the elderly differs from the mean protein requirement for younger adults; ie, 0.6 g/kg.

Two important studies have addressed the issue of whether variation in protein intakes toward marginal intakes is detrimental in elderly persons consuming self-selected diets. Munro et al (35), addressing in some extent the issue of both “how much” and “what for,” reported measurements of dietary intakes, plasma proteins, and arm muscle area for 691 men and women aged 60–98 y consuming on average 1.04 g protein/kg. Only 12–15% of subjects had protein intakes <0.8 g/kg (possibly reflecting inadequacies of 3-d records) but clear, overt protein deficiency was not observed. Nevertheless, there was no evidence that lower intakes of protein in the group adversely influenced any measured variable. Indeed, both arm circumference and a “nutritional index” score calculated from albumin, triceps skinfold thicknesses, and transferrin concentrations were inversely correlated with protein intakes, implying no deleterious effect of consuming protein at the lower end of the observed range.

Bunker et al (36) reported actual nitrogen balances for both homebound elderly people (n = 20 aged 70–86 y) with mean protein intakes of 0.67 g protein·kg⁻¹·d⁻¹ and mostly in negative balance, and for healthy men and women (n = 24, aged 70–86 y) with mean protein intakes of 0.97 g protein·kg⁻¹·d⁻¹ at zero balance overall. However, there was no indication that protein intake determined balance. There was no correlation between protein intake and balance in either group over a wide range of intakes: 24–79 g protein/d in the house bound and 35–92 g protein/d in the healthy group. Furthermore, at the same intakes homebound subjects tended to be in negative balance whereas the healthy subjects were in positive balance. Either the immobility, illness, or the lower energy intake of the homebound subjects accounted for the negative nitrogen balance. Notwithstanding the limitations of such measurements, the data do not support any effect of protein intake on nitrogen balance over a range of intakes as wide as that likely to be observed in a free-living population.

Thus, in the context of both “how much” and with “what for” limited to plasma proteins and body composition, these two studies both point to free-living elderly individuals being able to adapt to protein intakes over a wide range, with no benefit in terms of either biochemical indicators or measured balance from increased intakes. When Millward and Roberts (25) assessed specific nitrogen balance studies, they were unable to identify any convincing evidence for a revision of the 1985 FAO/WHO/UNU recommendations and concluded that there appeared to be no change with age in the protein requirement per kilogram body weight. Indeed, they raised the possibility that the actual biological demand for protein and amino acids in the elderly is lower than in younger adults.
AGE-RELATED CHANGES IN PROTEIN AND AMINO ACID TURNOVER

Assuming that protein requirements reflect the relative size of the fat-free mass (FFM), any decrease with age in the overall FFM might be assumed to result in a fall in protein requirements per kilogram body weight. Assuming further that protein requirements are related to the intensity of cellular metabolism in terms of protein turnover (37), then it becomes important to establish the extent of any change with age. In fact, given the lower rates of protein turnover in muscle compared with most nonmuscle tissues (38), any sarcopenia might be expected to minimize any fall with age in the overall rate. Indeed, there is a possibility of an increase in protein turnover per unit FFM. Changes in body composition in the direction of loss of skeletal muscle, as observed in chronically undernourished individuals, can result in increases in the resting metabolic rate per FFM obscuring any adaptive changes in specific tissues (39). If such changes also occur with respect to protein turnover, this could reduce the influence of any fall in turnover in a specific tissue on the overall whole-body rate.

There are several published reports on the extent of age-related changes in whole-body protein turnover. An early report of a somewhat limited study (40) suggested a considerable reduction but subsequent studies have not supported this. A summary of all studies we are aware of is given in Table 2. The studies vary considerably both in terms of methodology and design so that comparisons are difficult. The methods include the $[^{15}N]$glycine end product method, which assesses overall turnover (studies 1, 2, 4; 11-13); the $[^{15}N]$glycine flux, which includes rates of de novo glycine synthesis as well as protein turnover, and which enables albumin synthesis to be measured (study 3); the leucine precursor method, which assesses protein turnover (synthesis and proteolysis) and leucine oxidation (studies 2, 5-10), and skeletal muscle protein synthesis (studies 9 and 10). Some studies only involved the postabsorptive state (studies 5-7, 9-12), others were carried out only in the fed state (studies 8 and 13) or during prolonged periods of both feeding and fasting (studies 1-4), and one study (no. 14) only reported on elderly women in terms of the response of fed-state $[^{13}C]$leucine turnover and oxidation to a reduced protein intake. Finally, whereas some studies only reported comparisons on a body-weight basis (studies 2 and 8), all others attempted to account for body-composition changes in terms of FFM or skeletal muscle mass as indicated by creatinine excretion. As indicated in Table 2, in addition to examining the influence of age, other variables were examined, including sex (studies 1, 11, 12), response to protein intake (studies 3, 14) or glucose intake (study 5), response to protein intake before the infusion (studies 11 and 12), the influence of immobility (study 8), and physical training (study 10).

All studies reported a fall in overall rates of whole-body protein turnover per kilogram body weight (exception for study 13) in one or both sexes (studies 1, 2, 4, 8, and 12). However, after any change in body composition was corrected for by expressing the rate per FFM, no age-related change was apparent in any study apart from study 12, in which there is a decrease in women but not men. This tends to point to a decrease in turnover within one or more compartments compensating for any increase in the overall rate per kilogram FFM due to sarcopenia changing the relative amounts of "slow" and "fast" tissues.

A fall in skeletal muscle turnover does appear to occur. Thus, although there was no significant change with age in the urinary 3-methylhistidine--creatinine ratio (28), two other studies reported a marked fall in postabsorptive muscle protein synthesis (37, 50). Yaarashki et al (37) argued that the reduction in physical activity with age was responsible for this change after showing that resistance training fully restored protein synthesis to rates seen in younger adults. Alternatively, given that growth hormone normalizes skeletal muscle protein synthesis in aged rats (51) and increases skeletal muscle mass in elderly humans (52), hormonal changes with age cannot be ruled out as important components of the decline.

As to any influence of age on the other variables examined in the studies assembled in Table 2, in study 3 Gersovitz et al (43) showed that in the elderly, in contrast with the younger subjects, rates of albumin synthesis were not responsive to a change in protein intake and argued that this indicated a different regulatory set point for albumin synthesis. This conclusion was supported by the lack of any correlation between protein intake and albumin concentrations in the elderly (35). As for the effect of immobility on whole-body protein turnover, in study 8 this was somewhat surprisingly found to result in increased turnover (49). Finally, on the basis that previous diet may confound any age-related changes in protein turnover, in studies 11 and 12 Pannemans et al (29, 30) standardized dietary protein intakes before measurement, reporting that differences with age were dependent on the protein intake, falling when the dietary protein-energy ratio was 12% but only falling in women with a protein-energy ratio of 21%. These findings were unaltered by correction for body composition and indicate somewhat surprisingly higher turnover rates in elderly men than in elderly women, the opposite of what might be expected from a higher skeletal muscle mass. The significance of these findings is not immediately apparent. With the $[^{15}N]$glycine end product method, distinction between synthesis and proteolysis is more difficult to identify, requiring accurate and very short-term nitrogen balance measurements. This means that in studies such as this with turnover measured in the postabsorptive state, the relation between protein turnover in terms of replacement of the body protein pool and net loss, the nutritionally important variable, is difficult to assess. In addition Pacy et al (4) were unable to identify any influence of dietary intake on the mean daily rate of protein turnover over a very wide range of intakes. Similarly, Castaneda et al (31) investigated mechanisms of accommodation to reduced protein intakes (from 0.92 to 0.45g·kg$^{-1}$·d$^{-1}$) in elderly women in terms of fed-state leucine kinetics and observed reduced leucine oxidation but no changes in leucine turnover. This could reflect a methodologic difference with the work of Pannemans et al (29, 30) based on $[^{15}N]$glycine methodology and $[^{13}C]$leucine studies (4, 31).

Other studies of age-related changes that may be relevant to nitrogen homeostasis relate to insulin sensitivity. On the basis of the well-established progressive deterioration in glucose tolerance with advancing age (53) due, in part, to a decline in peripheral tissue sensitivity to insulin (46, 54, 55), Fukagawa et al (47) investigated whether this age-related defect in insulin action extended to effects on amino acid metabolism. The rationale for this was the well-established role of insulin in protein utilization (38,
### TABLE 2

Measurements of protein turnover in the elderly

<table>
<thead>
<tr>
<th>Study, reference, sex, and age</th>
<th>Tracer</th>
<th>Parameter</th>
<th>Variable</th>
<th>Finding</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. (41) 4M, 5F; 65–91 y</td>
<td>[13N]Glycine, oral, 60 h</td>
<td>Rate/kg body wt</td>
<td>Age</td>
<td>Slight fall</td>
</tr>
<tr>
<td>2. (42) 3M, 3F; 66–91 y</td>
<td>[13N]Glycine: [14C]Leucine</td>
<td>Rate/kg body wt</td>
<td>Age</td>
<td>Slight fall</td>
</tr>
<tr>
<td>3. (43, 44) 5M, 19–25 y</td>
<td>[13N]Glycine</td>
<td>Rate/kg FFM</td>
<td>Protein intake</td>
<td>No effect</td>
</tr>
<tr>
<td>4. (28) 6M, 5F; 67–91 y</td>
<td>[13N]Glycine, oral, 60 h</td>
<td>Rate/kg body wt</td>
<td>Age</td>
<td>Loss of sensitivity</td>
</tr>
<tr>
<td>5. (46) 6M, 4F; 73–79 y</td>
<td>[13C]Leucine</td>
<td>Rate/kg body wt</td>
<td>Age</td>
<td>Slight fall</td>
</tr>
<tr>
<td>6. (47) 5M, 21–34 y</td>
<td>[13C]Leucine</td>
<td>Rate/kg body wt</td>
<td>Age</td>
<td>No difference</td>
</tr>
<tr>
<td>7. (48) 6M, 21–25 y</td>
<td>[13C]Leucine</td>
<td>Rate/kg FFM</td>
<td>Age</td>
<td>No effect</td>
</tr>
<tr>
<td>8. (49) 3F, 6M, fit; 68–89 y</td>
<td>[13C]Leucine</td>
<td>Rate/kg body wt</td>
<td>Age (fit)</td>
<td>Fall</td>
</tr>
<tr>
<td>9. (50) 8M, 21–31 y</td>
<td>[13C]Leucine</td>
<td>Rate/kg FFM</td>
<td>Age</td>
<td>No effect</td>
</tr>
<tr>
<td>10. (37) 2M, 4F; 23–25 y</td>
<td>[13C]Leucine</td>
<td>Rate/kg FFM</td>
<td>Age</td>
<td>No effect</td>
</tr>
<tr>
<td>12. (30) 19M, 10F; 23–25 y</td>
<td>[13N]Glycine</td>
<td>Rate/kg body wt and FFM</td>
<td>Prior protein intake</td>
<td>Positive correlation</td>
</tr>
<tr>
<td>13. (45) 26 young</td>
<td>[13N]Glycine</td>
<td>Rate/kg body wt and FFM</td>
<td>Sex</td>
<td>M &gt; F</td>
</tr>
<tr>
<td>14. (31) 12F, elderly</td>
<td>[13C]Leucine</td>
<td>Rate/kg</td>
<td>Protein intake</td>
<td>Reduced</td>
</tr>
</tbody>
</table>

1. P:E, ratios of dietary protein to energy; IV, intravenously; FFM, fat-free mass; 3-MH, 3-methylhistidine.

56). With [1-13C]leucine infusion combined with the euglycemic insulin-clamp procedure Young (57) showed that in the postabsorptive state, insulin mediated the same inhibition of endogenous leucine appearance (proteolysis), and a similar pattern of changes in plasma amino acid concentrations (47, 58, 59) in both young and elderly subjects. On this basis, there are differential changes during aging in the sensitivities of glucose compared with amino acid metabolism to insulin action, and aging does not appear to bring about a deterioration in the anabolic influence of insulin on amino acid metabolism. Subsequent studies examining the relative roles of insulin and of amino acid availability on the responses of body protein synthesis in both young and elderly subjects showed that there were similar responses of whole-body leucine kinetics to the provision of amino acids in young and old subjects (48).

### PROTEIN REQUIREMENTS AND PROTEIN METABOLISM

The studies assembled in Table 2 suggest that although age-related changes in protein turnover in skeletal muscle
appear to occur, age-related changes in whole-body protein turnover are small, if they occur at all, and near the limits of detection by these methodologies. In any case, the relation between protein requirements and protein metabolism and turnover needs to be examined carefully. In fact, the first place the technical problems limiting the interpretation of isotopic studies are considerable (4, 60, 61). Second, most of the above investigations of the rate of protein turnover have been made mainly on the basis that this is a proxy for all metabolic activity that relates to the protein requirement. However, there is little evidence for, or indeed, reason why, protein requirements should reflect turnover as such. "Wea and tear," the oft-quoted main component of any protein turnover–related amino acid requirement, is not an appropriate analogy. Thus, apart from some posttranslational modifications of amino acids (eg, the 3-methylation of histidine), there is no evidence that amino acids liberated by proteolysis during protein turnover are any more or less susceptible to amino acid oxidation than are dietary amino acids, which have never participated in protein turnover. The general correlation between rates of protein turnover and endogenous nitrogen losses, in which rates of both processes change in relation to organism size and basal metabolic rate, and which is usually quoted as evidence of linkage between turnover, amino acid oxidation, and consequent requirements, has not been shown to be a causal link. It is more likely to be a simple reflection of the generally parallel metabolic changes in many cellular processes that make up the basal metabolic rate and contribute to both protein turnover and obligatory nitrogen losses. This means that the relation between protein requirements and protein metabolism needs to be approached from a different perspective.

Metabolic demand and efficiency of utilization
From first principles, protein requirements measured by nitrogen balance studies should reflect a combination of the metabolic demand for protein and the efficiency of its utilization from food. For adults in balance the protein requirement for nitrogen homeostasis will be influenced by both the magnitude of losses of amino acids and nitrogen from the body throughout the day by whatever route, which generates the metabolic demand, and the efficiency of dietary protein utilization to replete these losses. In this case, the relation between the protein requirement and protein turnover is more easily identified in terms of the regulatory responses of tissue protein synthesis, proteolysis, and amino acid oxidation to feeding and fasting in response to the habitual protein intake.

Recently, a new metabolic framework was described that allows the systematic examination of the protein requirement and turnover within the context of the diurnal cycle of losses and gains (60, 62, 63), and this has been applied to the effects of aging in both men and women (64). The key feature of the model is that of an adaptive metabolic demand for protein that is variable according to the habitual protein intake, which in turn sets amino acid oxidation throughout the day (60, 63) at rates that will occur regardless of the acute protein intake (65). Thus, healthy individuals will exhibit an apparent protein requirement for balance that is determined by a metabolic demand, mainly generated in response to their habitual protein intake, and an efficiency of postprandial protein utilization. Inherent in this approach is the assumption that normal healthy individuals can, with time, safely adapt their metabolic demand to match their protein intakes over a wide range of protein intakes down to amounts considerably below those habitually consumed by individuals satisfying their energy and micronutrient requirements with mixed diets. Such an intake, which can be defined as the minimal protein requirement for lean body mass maintenance, is only measurable in long-term balance studies and its magnitude is poorly understood and arguably irrelevant in the context of practical nutrition. Certainly, as indicated above for the elderly, no such studies have been reported. What can be measured instead is whether in healthy adults aging per se influences the characteristics of nitrogen homeostasis and turnover in a way that might influence their nutritional needs. For example, if the metabolic demand at usual dietary protein intakes increased in the elderly, there would be a need for an increased efficiency of protein utilization if balance was to be maintained with intakes similar to those of younger adults. This in turn would mean that meal timing and composition, which could influence postprandial protein utilization, would be more critical in the elderly.

Calculation of metabolic demand and efficiency of utilization from [13C]leucine balance studies
We have begun to explore metabolic demand and efficiency of utilization in healthy elderly men and women by means of a novel stable isotope[13C]leucine balance technique (64). The calculation of the efficiency of postprandial protein utilization, metabolic demand, and protein requirements is based on the assumption that the metabolic fate of [13C]leucine can be assumed to trace the overall utilization of amino acids in all metabolic pathways, including metabolic consumption, catabolism, and protein utilization. Thus, leucine turnover and balance is measured during a 9-h constant intravenous infusion of [1-13C]leucine in subjects initially in the postabsorptive state (3 h) and then sequentially fed frequent small low-protein (3 h) and then high-protein meals (3 h) at intervals that match the subjects’ habitual protein intake. This protocol allows leucine balance to be measured at three leucine intakes so that the efficiency of postprandial protein utilization can be calculated from the leucine balance curve (62). In addition, whole-body rates of protein synthesis and proteolysis are indicated by the leucine turnover data, enabling both the regulatory responses of protein synthesis and proteolysis to feeding and fasting to be studied, as well as calculation of an average rate of overall protein turnover throughout each 24-h daily cycle from the rates measured in the fasting and high-protein feeding periods.

In these studies metabolic demand is defined in relation to postabsorptive [13C]leucine oxidation, which is assumed to trace overall amino acid consumption in all irreversible metabolic transformations, including both obligatory and adaptive components, which ultimately results in amino acid oxidation and nitrogen excretion. On the basis of diurnal [13C]leucine balance studies (63, 65) this can be assumed to occur at a constant rate throughout each daily cycle. Therefore, metabolic demand is simply defined as postabsorptive leucine oxidation scaled to a rate per 24 h. This can be expressed in terms of protein: ie, with the leucine content of tissue protein at 3.93 mmol/g N (63) the protein equivalent of postabsorptive leucine oxidation is 0.629 mmol/g protein.

In the fasted state, tissue protein provides for metabolic demand whereas during feeding metabolic demand is provided for by dietary protein. For overall daily balance dietary protein
must also provide for the repletion of postabsorptive protein lost in satisfying the metabolic demand in the postabsorptive state. In this way, dietary protein provides for both postprandial and postabsorptive metabolic demands.

The efficiency of postprandial protein utilization (PPU) is determined by the extent of any increased amino acid oxidation on feeding, i.e., oxidation additional to the metabolic demand. It can be calculated from the increase in leucine oxidation with feeding, which will determine the slope of the leucine intake–balance curve in terms of the entire meal (both energy and protein), from postabsorptive to high protein intake (PPU ᵐᵉᵃˡ). PPU ᵐᵉᵃˡ, the efficiency of protein utilization solely in terms of the protein intake, is indicated from the slope calculated between the low protein and high protein intakes. Because the main part of the change in leucine balance occurs during the low-protein to high-protein transition, PPU ᵐᵉᵃˡ will not be expected to differ markedly from PPU ᵐᵉᵃˡ.

The apparent protein requirement is the intake required to satisfy the metabolic demand, taking into account any inefficiency of utilization that will result in an increased rate of postprandial leucine oxidation compared with the postabsorptive rate. Therefore,

Apparent protein requirement = metabolic demand/PPU

Because any age-related changes in PPU could involve responses to both energy (eg, insulin) and protein (amino acids), the protein requirement is best calculated from PPU ᵐᵉᵃˡ.

This protocol was applied to 25 subjects shown in Table 3: young women and men, middle-aged men, and elderly women and men, all in good general health with normal renal and hepatic function. The elderly subjects were all mobile. Body composition was assessed by underwater weighing and bioelectrical impedance. The results for metabolic demand and efficiency of protein utilization for the 20 young and elderly subjects were published recently (64) and are shown here together with the additional data for the middle-aged men.

The values calculated for metabolic demand, the efficiency of PPU, and the apparent protein requirement are shown in Table 4. In both elderly groups the metabolic demand was markedly reduced, either per kilogram body weight or per kilogram FFM, compared with the younger control subjects, whereas the values for the middle-aged men were similar to those for the young men. As for PPU, although values were lower in women than in men, with the lowest mean values in the elderly women, there was no significant overall age effect.

With a lower metabolic demand in the elderly and with no change in the efficiency of utilization, the apparent protein requirement calculated from these values was also lower in the elderly on the basis of body weight and FFM. The individual values for the apparent protein requirements as a function of age are shown in Figure 1 per kilogram FFM; there was a significant inverse correlation (r = −0.55, P = 0.005).

These are the first data of their kind and although it is widely assumed that there may be a reduced efficiency of protein utilization (1), in fact, the evidence for this has been scant. Thus, in contrast with the popular view that the elderly have a higher protein requirement than younger adults, these studies indicate that the opposite of this appears to be the case at least in terms of an apparent protein requirement calculated from leucine balance studies as described here. Although the age-related decline in the apparent requirement is shown as a linear function in Figure 1, in fact, it is by no means clear whether this is the case, as indicated by the additional values for the middle-aged men. Thus, regression of all values for subjects aged < 60 y indicates that neither metabolic demand nor apparent requirement varied with age (P > 0.9).

As with all measures of nitrogen homeostasis and balance, these studies involve many assumptions relating to calculation of leucine oxidation rates; i.e., the relations between leucine balance as measured during the postabsorptive phase compared with the entire postabsorptive balance, between leucine balance and protein balance, and between efficiency of PPU as measured and that exhibited during usual meal consumption. The limitations and potential errors flowing from our assumptions and calculations are discussed extensively elsewhere (64). The kinetic assumptions and isotope-related problems associated with [¹³C]leucine tracer balances have also been reviewed extensively (60, 61, 66). They include the effects of the true precursor enrichment, the possibility of tracer recycling, and the splanchnic sequestration of dietary protein (the first-pass effect). Although a recent study suggested an increased first-pass effect in the elderly (67), when this report is examined in detail it would appear that this is unlikely to be a generally serious issue. In this study (67), the splanchnic sequestration of leucine in the elderly was linearly related to their body mass indexes (BMIs; in kg/m²) with marked increases only observed in subjects with high values (eg, 27-30), possibly reflecting an influence of body composition, especially an increased splanchnic organ mass, which usually accompanies an increased BMI. In the studies of Fereday et al (64), whereas the

### Table 3

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Age (y)</th>
<th>Weight (kg)</th>
<th>Height (m)</th>
<th>FFM (kg)</th>
<th>Fat (%)</th>
<th>Body wt (kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>26.0 ± 3.8</td>
<td>56.9 ± 2.2</td>
<td>1.70 ± 0.05</td>
<td>47.1 ± 4.1</td>
<td>17.3 ± 4.5</td>
<td></td>
</tr>
<tr>
<td>F2</td>
<td>72.0 ± 4.6</td>
<td>61.4 ± 6.2</td>
<td>1.59 ± 0.08</td>
<td>45.4 ± 5.0</td>
<td>26.0 ± 4.0</td>
<td></td>
</tr>
<tr>
<td>M1</td>
<td>21.4 ± 1.4</td>
<td>67.3 ± 3.1</td>
<td>1.76 ± 0.04</td>
<td>58.6 ± 2.6</td>
<td>13.0 ± 3.3</td>
<td></td>
</tr>
<tr>
<td>M2</td>
<td>43 ± 10.0</td>
<td>73.5 ± 13.3</td>
<td>1.74 ± 0.07</td>
<td>56.8 ± 6.3</td>
<td>21.9 ± 7.3</td>
<td></td>
</tr>
<tr>
<td>M3</td>
<td>77.6 ± 8.1</td>
<td>71.4 ± 7.7</td>
<td>1.72 ± 0.03</td>
<td>59.4 ± 5.7</td>
<td>15.3 ± 5.8</td>
<td></td>
</tr>
</tbody>
</table>

1 Data as presented in reference 65 except for group M2 (middle-aged men), which is additional data. n = 5 per group. F1, young females; F2, elderly females; M1, young males; M3, elderly males.

There were significant differences by two-way ANOVA (age and sex): weight × sex, P = 0.002; height × age, P = 0.02; height × sex, P = 0.001; FFM × sex, P ≤ 0.001; fat × sex, P = 0.05; interactions, P = 0.05.
TABLE 4
Metabolic demand, efficiency of postprandial protein utilization (PPU), and apparent protein requirementa

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Metabolic demand</th>
<th>Apparent protein requirement</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Per weight</td>
<td>Per FFM</td>
</tr>
<tr>
<td></td>
<td>g · kg⁻¹ · d⁻¹</td>
<td>g · kg FFM⁻¹ · d⁻¹</td>
</tr>
<tr>
<td>F1</td>
<td>0.83 ± 0.15</td>
<td>1.01 ± 0.21</td>
</tr>
<tr>
<td>F2</td>
<td>0.52 ± 0.16</td>
<td>0.71 ± 0.21</td>
</tr>
<tr>
<td>M1</td>
<td>0.90 ± 0.06</td>
<td>1.03 ± 0.11</td>
</tr>
<tr>
<td>M2</td>
<td>0.87 ± 0.18</td>
<td>1.12 ± 0.23</td>
</tr>
<tr>
<td>M3</td>
<td>0.58 ± 0.19</td>
<td>0.69 ± 0.21</td>
</tr>
<tr>
<td>All</td>
<td>0.74 ± 0.21</td>
<td>0.91 ± 0.26</td>
</tr>
</tbody>
</table>

aData as presented in reference 65 except for group M2 (middle-aged men), which is additional data. n = 25 total; n = 5 per group. F1, young females; F2, elderly females; M1, young males; M3, elderly males.

There were significant differences by two-way ANOVA (age and sex): metabolic demand: per weight × age, P ≤ 0.001; per FFM, P ≤ 0.001; efficiency of PPU × sex, P ≤ 0.02; apparent protein requirement: per weight × age, P ≤ 0.01; per FFM, P ≤ 0.01.

mean BMI of the elderly subjects (24.4 ± 2.4; n = 10) was on average greater than for the other subjects (21.9 ± 2.1), the differences were only significant for the women (24.2 ± 2.9 for the elderly and 19.8 ± 1.05 for the young women) and even then the magnitude of the differences was small. Thus, within the limitations of the experimental model there is little reason to believe the results in Table 4 to be seriously in error.

Any measurement of the elderly must take into account the fact that within any given age range there is considerable heterogeneity of physiologic state. The relatively small number of subjects examined in these recent studies represented a wide age range (68–91 y) recruited from an overall healthy and mobile population and there was in fact little evidence of extensive sarcopenia because on the basis of the height of the subjects, FFM was not reduced (64). However, cross-sectional comparisons such as these studies reveal little about previous body size and composition. Furthermore, with the shrinkage of height that occurs with age it is possible that FFM-height ratios can increase with age so that these elderly subjects could have represented an initially larger group who had nevertheless lost FFM and muscle mass. In any case, measurement of body composition in the elderly is difficult (68). Densitometry as used in these studies requires assumptions about the density of the FFM and because this includes bone mineral mass, actual density will vary with bone mass and mineralization (69).

In the elderly persons described here there was a wider range of values for the metabolic demand than in the younger subjects. However, there were no anthropometric or biochemical correlates of this variability. Overall nitrogen balance varies from day to day, so that on any one day excretion may underestimate intake and the daily CV can be as high as 20% (70). It has yet to be determined whether there is day-to-day variability in postabsorptive leucine oxidation, and hence the calculated metabolic demand, and whether this variability increases in the elderly. As for the decrease in metabolic demand with age, the magnitude is much greater than could be accounted for in terms of age-related changes in the miscellaneous obligatory nitrogen losses, because the decrease shown in Table 4 is equivalent to the entire obligatory loss.

It may be that the ability to maintain protein balance in the postabsorptive state is variable between tissues so that changes in the relative amounts of skeletal muscle and visceral tissue with age could influence overall metabolic demands. In humans the relative losses of tissue protein from different tissues in the postabsorptive state is not known, but in animals short-term losses from visceral tissues with fasting are generally more rapid than from skeletal muscle (38). With sarcopenia therefore, an increased postabsorptive loss per kilogram FFM might be expected—the opposite of that observed.

FIGURE 1. Apparent protein requirements as a function of age. The line is the linear regression of the apparent protein requirement on age (r = −0.55, P = 0.005; n = 25).
Postabsorptive protein turnover and metabolic demand

Within the Fereday et al model (64) differences in the metabolic demand reflect differences in net loss of body protein in the postabsorptive state and this must reflect differences in the balance between protein synthesis and proteolysis. Given the significantly lower metabolic demand in both elderly men and women per kilogram or per kilogram FFM, indicating a lower rate of net proteolysis, the mechanism for the effect of age could be either a better maintenance of protein synthesis against the prevailing rate of proteolysis or vice versa, a more restrained rate of proteolysis in relation to the prevailing rate of protein synthesis. In fact, as shown by the plot of postabsorptive rates of protein synthesis and proteolysis per kilogram FFM with age in Figure 2, only proteolysis showed a significant correlation, falling with age with a slope that was twice that of synthesis. This would indicate that the main reason for the lower net proteolysis in the postabsorptive state with age was a lower rate of proteolysis at the prevailing rate of protein synthesis, which did not fall significantly with age.

As far as we are aware, this is a novel finding because no other studies have addressed this specific question. However, the finding is to some extent similar to that from the study by Jeeranandam et al (71) on the responsiveness of whole-body protein and glucose kinetics to severe trauma in elderly patients. They noted that geriatric trauma patients without any nutritional support lost less nitrogen (in g/d) than younger patients, mainly because of a significantly decreased whole-body protein breakdown rate, a response that reflected both age and reduced FFM. Why postabsorptive proteolysis should be better restrained in the elderly is not known. If there were changes in the relative proportions of skeletal muscle and visceral tissues in the elderly, coupled with differences in the way in which proteolysis is regulated in the postabsorptive state between these two components of the FFM, then this could afford an explanation. However, as discussed above there was little evidence of specific muscle wasting in the elderly described by Fereday et al (64). In any case, although some have suggested between-organ differences in amino acid recycling (72), there is no substantive evidence that the visceral tissues are better able to maintain balance in the postabsorptive state than is muscle.

Postprandial leucine turnover and protein utilization

The efficiency of PPU is determined by the changes in rates of oxidation, protein synthesis, and proteolysis with feeding. Gibson et al (73) identified the pattern of responses to the three-phase feeding regimen used by Fereday et al (64). They showed that the feeding of isoenergetic, small, frequent low-protein and then high-protein meals maintains a constant physiologic insulin concentration, which allows the effects of protein feeding to be separately evaluated from the insulin-mediated responses to energy. The feeding mechanism involves both an insulin-mediated, protein-conserving influence of dietary energy, inhibiting proteolysis, lowering amino acid concentrations and reducing amino acid oxidation, and an amino acid-mediated augmentation of the inhibition of proteolysis, which adds to the insulin-mediated inhibition, together with an amino acid-mediated stimulation of protein synthesis.

In fact, analysis of the relative effect of the changes in proteolysis and protein synthesis on the efficiency of protein utilization in these elderly and younger subjects showed clearly that PPU was mainly a function of inhibition of proteolysis rather than stimulation of protein synthesis. Thus, not only was the overall magnitude of the inhibition of proteolysis by feeding greater than the stimulation of synthesis, but PPU varied inversely with proteolysis (high-protein diet; \( r = -0.498, P = 0.01 \)) and was not significantly correlated with protein synthesis (high-protein diet; \( r = -0.18, P = 0.39 \)). However, no component of the responses to feeding that changed with age was identified that related to the rate or the regulation of protein turnover by insulin or amino acids. This is consistent with the finding of no relation between the metabolic demand and the efficiency of PPU, which was not significantly different in this elderly cohort. Other studies have also failed to identify any change with age in the sensitivity of either protein synthesis or proteolysis to feeding consistent with these findings. Young et al (72) examined the response of plasma amino acids to graded intakes of leucine, tryptophan, and valine and

![Figure 2](https://academic.oup.com/ajcn/article-abstract/66/4/774/4655904/6655901)

**Figure 2.** Postabsorptive rates of protein turnover, metabolic demand, and age. Postabsorptive protein turnover (degradation, ■), and synthesis (○), is shown with metabolic demand (●). The lines are linear regressions of proteolysis (solid line: \( r = -0.364, P = 0.074 \)), protein synthesis (dotted line: \( r = -0.525, P = 0.007 \)), and metabolic demand \( (r = -0.645, P = 0.001) \) with age.
reported no age effects. Fukagawa et al (47) were unable to identify any age-related change in the insulin-mediated suppression of whole-body protein breakdown and changes in plasma amino acid concentrations in [13C]leucine, euglycemic insulin-clamp studies in the postabsorptive state, or in the early postprandial state (48).

These new findings of no decrease in the efficiency of PPU with age are, however, contrary to one previous proposal relating to changes in the distribution of protein turnover in the body with age (72). It was argued in this report that if the major body organs have different requirement patterns for amino acids and different efficiencies for their recycling, a shift in the distribution of whole-body protein metabolism with aging from muscle to viscera may result in changes in the efficiency of dietary protein utilization. On the other hand, an accompanying suggestion that a reduced muscle mass and skeletal muscle protein turnover may reduce the ability of the elderly to respond successfully to dietary or other stresses that require adaptive changes in muscle protein and energy metabolism (72) is to some extent consistent with our observed lower postabsorptive loss and the findings with elderly trauma patients reported above (71). What is not known is whether the better restraint of proteinolysis in the postabsorptive state benefits the elderly.

**Daily protein turnover and the apparent protein requirement**

Protein turnover involves protein degradation and resynthesis and with both rates varying in response to feeding and fasting, care is needed to define it unambiguously when discussing its physiologic regulation and significance. In these studies a "mean daily rate" of protein synthesis and proteolysis was calculated from postabsorptive and postprandial rates during the high-protein phase, assuming equal time spent in these states, and these values give a measure of actual turnover or replacement during each diurnal cycle. Identification of any age-related change in protein turnover in which turnover is defined as replacement requires that either protein synthesis or proteolysis is identified as the best indicator of the replacement rate. In fact, because the main mechanism of change in balance during feeding or fasting is a change in proteinolysis, which always appears greater than any change in protein synthesis, this identifies protein synthesis as the best indicator of replacement (nearer to the value obtained in the steady state). As indicated in Figure 3, protein synthesis per kilogram FFM did not significantly change with age, \( r = -0.27, P = 0.18 \) so that on this basis protein turnover did not significantly change with age. In fact, proteinolysis fell by \( \approx 20\% \) over the age range examined \( r = -0.40, P = 0.048 \) but these changes with age mainly reflect the lower concentrations in elderly women than in young men and there were in fact no significant age or sex differences according to analyses of variance.

Thus, for these mobile elderly subjects with little evidence of loss of lean body mass, the extent of any age-related fall in protein turnover per unit FFM was small when compared with younger subjects consuming similar habitual protein intakes (64). This is broadly consistent with the previous studies reviewed in Table 2, although none of the previous studies allow calculation of a mean daily rate in this way.

As for the relation between daily protein turnover and the apparent protein requirement, because this latter measure is calculated from the leucine balance data, the metabolic demand divided by efficiency of protein utilization, then it should vary with the daily protein turnover rate to the extent that postabsorptive protein turnover influences the metabolic demand and postprandial protein turnover is related to PPU. As discussed above, the metabolic demand varied directly with postabsorptive proteinolysis. This relation also means that metabolic demand also varied directly with the daily rate of proteinolysis \( r = 0.458, P = 0.021 \). As also discussed above, PPU varied inversely with proteinolysis (high-protein diet) and as a result PPU varied inversely with the daily rate of proteinolysis, although not significantly \( r = -0.305, P = 0.138 \). Because the apparent protein requirement = metabolic demand/PPU, with a lower daily rate of proteinolysis associated with a lower metabolic demand and higher PPU, then a marked relation between daily proteinolysis and the apparent protein requirement would be expected. In fact, this was observed \( r = 0.59, P = 0.002 \) for proteinolysis/kg FFM. In contrast, the correlation of the requirement with daily protein synthesis was not significant \( r = 0.32, P = 0.17 \) for protein synthesis/kg FFM. Thus, individuals with the lowest daily rate of proteinolysis had the lowest apparent protein requirement when it was calculated this way.

**Figure 3.** Daily rates of protein turnover as a function of age. Daily rates of protein synthesis (■) and degradation (□) were calculated from rates measured in the postabsorptive and high-protein periods scaled to 24 h. The lines are linear regressions of degradation (solid line: \( r = -0.40, P = 0.048 \)) and protein synthesis (dotted line: \( r = -0.27, P = 0.18 \)) plotted against age.
This finding of a relation between protein turnover and the apparent protein requirement has often been assumed as discussed above but has not previously been shown and needs to be evaluated by taking into account the limitations and implications of this metabolic model. The plot of the daily rate of protein synthesis and proteolysis against the apparent protein requirement (Figure 4) shows that for all subjects protein synthesis was greater than proteolysis as a result of overall positive leucine balance. This does not mean that all subjects were gaining protein at their normal dietary intakes, it indicates only that there was a greater postprandial gain compared with the postabsorptive loss during the experimental protocol.

The protein requirement as calculated here is a function of the extent of postabsorptive loss and the efficiency of PPU. For the subjects as a whole intakes did not vary much so that it would be predicted that the extent of daily leucine balance would be inversely related to the requirement. In other words, in a group of subjects fed similar intakes, those with lower apparent protein requirements would achieve more positive protein balance within the protocol. This is why the daily leucine balance was highly inversely correlated with the apparent requirement ($r = -0.80, P < 0.001$). The implications of this as far as understanding the relations between daily rates of protein synthesis, proteolysis, and the apparent protein requirement is that it identifies which of the two processes is the main regulatory mediator of overall daily balance. Because protein synthesis did not vary significantly with the requirement, it would appear that daily turnover per se, ie, the replacement rate, did not vary with the apparent protein requirement and that changes in daily balance are mediated by changes in proteolysis.

However, this leads to the second issue, which is that because in all cases daily protein synthesis exceeds daily proteolysis, this protocol resulted in an overall positive leucine balance calculated from postabsorptive and postprandial leucine oxidation rates during the high-protein phase. Thus, the fall in proteolysis with age means that overall "daily" balance becomes more positive with age (mainly because the elderly exhibited a less negative postabsorptive balance). The fact of an overall positive balance in this protocol raises the question of the security of these derived values for an apparent protein requirement. In fact, because in the feeding phase the objective was limited to measuring the efficiency of utilization in a standardized protocol involving frequent small meals at intakes that corresponded to habitual diets, a positive hourly balance during the high protein phase that exceeded that required to balance postabsorptive loss on an hourly basis is not inconsistent with the model assumptions. As discussed by Fereday et al (64), whereas measured postabsorptive balance calculated from the leucine oxidation rate is likely to be representative of the daily rate of obligatory and habitual diet-related losses, ie, the metabolic demand, a necessary assumption within this model, there is no assumption that the gains measured during this protocol represent an hourly rate that has a simple relation with overall daily postprandial gain. It is only assumed that the protocol allows the efficiency of utilization to be measured in a standardized way and accurately identifies any differences between individuals. Thus, although the "apparent protein requirement" as measured here is arguably closely related to the actual operational requirement of the individual, it is not necessarily identical. Indeed, the fall with age in the apparent protein requirement (Figure 1) in subjects with similar habitual protein intakes implies that the elderly should be in positive balance. An explanation for this might be that for those few elderly with a lower apparent requirement, our estimation of their efficiency of utilization overestimated their actual efficiency during their actual daily feeding. Clearly, the next step within this sort of research is examination of the efficiency of protein utilization during actual meal feeding.

**CONCLUSIONS**

At the outset we posed the questions of whether the conclusions reached by the 1985 FAO report on protein requirements for the elderly remain valid today and whether measurements of age-related changes in protein and amino acid turnover afford any additional insight into the nutritional needs of the elderly for protein. As far as the requirement for balance is concerned the situation seems clear. The data in Table 1 and the arguments presented previously (25)—of no convincing evidence for any revision of the 1985 recommendations—are
supported by the new $[^{13}\text{C}]$leucine balance approach to evaluating how balance is achieved throughout the day (64). These new data indicate that in healthy elderly individuals there is a lower metabolic demand for dietary protein and on average a lower apparent protein requirement than in younger subjects because of apparent improvements rather than worsening in the control of protein balance in the postabsorptive state.

As to the issue of "protein requirement for what?" current knowledge of age-related changes in protein metabolism and turnover afford only limited insight into the issue of functional ability and nutritional status in the elderly. Thus, at the whole-body level, most age-related changes in protein turnover appear to be subtle and generally advantageous, such as the improved restraint of proteolysis in the postabsorptive state, which contributes to the apparent decrease in metabolic demand. The obvious exception is the decrease with age in protein turnover in skeletal muscle, which can be assumed to contribute to sarcopenia, loss of muscle strength, and metabolic reserve capacity in times of stress. However, this appears to be strongly related to lifestyle factors such as physical activity, especially resistance activities, and it is not yet clear how protein intake will interact with this and whether, for example, an increasingly active lifestyle will increase the protein requirement. We know of no studies with adequate experimental designs that offer unambiguous information on this. Similarly, the implications of immobility on the protein requirements of the elderly remain to be researched. As discussed elsewhere (74), exercise improves the efficiency of protein utilization in young adults so that in the immobile elderly the efficiency of protein utilization may indeed be decreased. It is to be hoped that some of the new approaches described will enable extension of our knowledge in this area.

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