Postprandial Protein Utilization: Implications for Clinical Nutrition

D. Joe Millward

Centre for Nutrition and Food Safety, School of Biomedical Sciences, University of Surrey, Guildford, Surrey, UK

The importance of enteral nutrition, especially for critically ill patients, is now widely recognized. There are clear benefits compared with parenteral nutrition in terms of reduced septic morbidity resulting from preservation of gut barrier function, not to mention reduced costs. Furthermore a more rational approach to feed formulation is emerging, with both energy and protein supply based on more scientific estimates of requirements [1–3], compared with the overestimates of needs commonly made in many centers in the past. In the case of protein, the objective is to minimize or prevent losses of the lean body mass, and ideally to maintain balance in the severely ill patient and to replete losses of tissue protein whenever possible. Thus the issue of effective protein utilization is a key part of any clinical nutrition strategy. However, because of the difficulties in conducting balance studies, especially in the environment of an intensive therapy unit, and with few centers attempting them, our knowledge of protein nutrition remains fragmentary.

My purpose in this chapter is to review the process of protein utilization from the perspective of acute postprandial measurements, examining how acute $^{13}$C-leucine balance studies can be used to assess the extent and regulation of postprandial protein utilization (PPU). These studies are based on simple principles of predicting N balance from leucine balance, and calculating the efficiency of PPU as an alternative to net protein utilization (NPU). The current database is small and restricted to studies in normal subjects. However, the methods which have been developed in these studies are eminently suitable for application in a clinical setting. We have previously described both the principles involved [4] and some of the results that we have obtained [5–9]. However, as with all novel tracer-based metabolic balance studies, the optimal methods
of data analysis can emerge as experience is gained in applying the methods. Thus in this chapter, as I will describe, we have revised some of our previous estimates.

**The Metabolic Model**

In humans, nitrogen balance is achieved against a background of a daily cycle of feeding and fasting [10–13]. This means that in adults maintaining overall balance there can be substantial postprandial protein deposition. The interesting feature of this phenomenon is that in healthy adults the amount of postprandial protein deposition in tissues can vary considerably, through adaptive changes in protein and amino acid metabolism in response to alterations in both acute and chronic dietary protein intake. For example, the amplitude of the diurnal gains and losses increases with the habitual protein intake [14]. Thus subjects with increased protein intakes have higher rates of amino acid oxidation and nitrogen loss throughout each 24-hour cycle, and this means that a greater postprandial gain is required to balance the greater fasting losses.

The most likely explanation for this phenomenon is an adaptive increase in the activity of key amino acid oxidation enzymes in response to the increasing dietary amino acid load [15], which cannot be switched off completely in the postabsorptive state. This has led us to describe human protein and amino acid requirements by a model in which dietary protein is utilized to provide for a metabolic demand that is best viewed as comprising two component parts (Fig. 1) [15, 16]. The first is the obligatory need for amino acids to balance the specific irreversible consumption of amino acids needed for various biosynthetic activities – including the production of catecholamines, serotonin, creatine, and so on – and lost in proteins secreted in skin, hair, and nails. As far as we know this part is best viewed as a fixed component. The second part is an adaptive component of oxidative loss that occurs in both postprandial and postabsorptive states. These two continuous pathways of amino acid consumption represent the metabolic demand. The metabolic demand for protein in subjects in overall balance involves metabolic consumption of amino acids by both obligatory and adaptive pathways occurring throughout the day. During fasting, body protein provides for the metabolic demand. During feeding the diet supplies the daily (24-hour) metabolic demand by providing for both the postprandial component of the metabolic demand and the repletion of tissue protein lost during fasting – that is, for the postabsorptive metabolic demand. The daily metabolic demand can be calculated as 24 times the hourly postabsorptive loss, which is the same as the sum of net protein gain and endogenous and adaptive oxidation in the postprandial state in subjects in overall balance. In addition the diet will be the source of any additional losses caused by inefficient utilization. The adaptive components vary markedly with dietary intake and change only slowly with intake [17].
Postprandial Protein Utilization: Implications for Clinical Nutrition

Fig. 1. Adaptive and obligatory metabolic demand for amino acids throughout the fasting and feeding cycle. Schematic changes in nitrogen balance during the daily feed/fast cycle [adapted from references 15 and 16]. Each protein meal converts the premeal negative balance into a positive balance. During fasting body protein provides for both obligatory and adaptive demands. During feeding the diet provides for obligatory and adaptive demands, for repletion of tissue protein lost during fasting and for any increased losses owing to inefficiency of utilization. The adaptive components vary markedly with dietary intake [14] and change only slowly with intake (17).

In the context of such a model the efficiency of PPU is determined by the partition of dietary amino acids between net protein deposition, oxidative catabolism that is part of the metabolic demand, and oxidative catabolism that is meal-related and represents the inefficiency. This means that the practical problem of measuring efficiency becomes that of identifying the size of the latter component. As discussed below, there are several ways to do this from the measured amino acid balance before and during feeding. Although careful measurements of nitrogen balance over 12 h can reveal information about the efficiency of PPU [14], stable isotope tracer balance studies afford the best method of measuring amino acid balances during feeding, and [13C-1]-leucine is most useful. With [13C-1]-leucine, leucine oxidation can be measured from 13CO2 excretion and from the enrichment of plasma α-ketoisocaproate, the precursor for leucine oxidation. With the exception of valine and isoleucine, no other amino acids afford such advantages. All the studies described here involve continuous intravenous infusion of trace amounts of [13C-1]-leucine.
Postprandial Protein Utilization: Implications for Clinical Nutrition

**Measurement of Postprandial Protein Utilization from [13C-1]-Leucine Balance**

Calculation of leucine tracer balance is uncomplicated kinetically, requiring only an accurate measure of leucine oxidation. For an indispensable amino acid like leucine, with a small, highly regulated free pool, if the intake and oxidative loss are measured accurately then the balance of that amino acid will be leucine in tissue protein, in most cases. Thus by measuring leucine oxidation and balance (oxidation less tracer) in the postabsorptive state, the metabolic demand can be calculated, and by measuring leucine balance during feeding, the efficiency of PPU (\(\Delta_{\text{balance}}/\Delta_{\text{intake}}\)) can be calculated. In our previous work we have calculated PPU directly from the leucine balance data. However, leucine balance does not predict protein or nitrogen utilization when the leucine content of the meal protein differs from tissue protein. An example of this is milk which is relatively leucine-rich (4.89 µmol leucine/mg N) compared with tissue protein (3.93 µmol leucine/mg N), and wheat, which is relatively leucine poor (3.21 µmol leucine/mg N). Thus when all milk protein is deposited some excess leucine will remain to either expand the free pool or be oxidized, and so leucine balance will underestimate milk protein balance. For wheat, the opposite is the case – that is, all of the leucine could be utilized with no increased oxidation when protein utilization is <100%. To allow for these differences the leucine intake and balances (leucine gain or loss) can be converted to nitrogen on the basis of leucine/N conversion factors. Thus the metabolic demand is calculated from postabsorptive leucine balance, expressed as nitrogen (3.93 mmol leucine/g N) [14], and PPU can be calculated from values for (\(\Delta N_{\text{balance}}/\Delta N_{\text{intake}}\)).

In the past, measuring protein utilization in humans has been part of the procedure for evaluating protein quality or defining protein requirements. It has usually involved feeding diets of varying protein concentrations for short balance periods and calculating NPU from the overall balance/intake slopes obtained with all the diets used. The slopes of such curves are always considerably less than 1, even for high-quality animal protein sources which, in animal studies, may be utilized with an efficiency of near 100%. In fact, in the context of a diurnal adaptive model of balance as in Figure 1, the NPU value obtained in the studies varies in a complex way with the actual PPU of each intake level, and is influenced by the extent of amino acid catabolism in both postprandial and postabsorptive phases of the cycle. In practice, in a clinical environment what is needed is information about the meal protein utilization for each meal in relation to the actual metabolic demands of the patient at the time, as this will determine the apparent protein requirement – that is, metabolic demand/PPU. While it may not always be possible to devise a protocol exactly matching the meal feeding pattern, it is possible to devise standardized protocols that allow reasonable estimates of this to be made. To date these studies have only involved healthy volunteers, but the methods described below, involving either
fasting/feeding protocols or two-stage feeding protocols with a low and then a high protein intake, are all eminently suitable for the enterally fed hospital patient.

**Effects of Meal Protein Concentration on Postprandial Protein Utilization**

Reports on animal studies show that the efficiency of protein utilization (NPU) is low at low protein concentrations, increasing to a maximum at about 10–15% energy and falling off at concentrations above this [18]. However, this relates to the way dietary protein influences overall lean tissue growth and is not physiologically comparable to postprandial repletion of postabsorptive losses. We have reported both N and $[^{13}\text{C}]$-l leucine balance studies with meal feeding of increased protein concentrations over a wide range [14] in subjects habituated to diets of increasing protein concentrations. We can examine these data in terms of the influence of meal protein concentration on PPU.

Figure 2A shows in schematic form the feed/fast $[^{13}\text{C}]$-leucine balance protocol employed in these studies [14]. The assumption is made that both obligatory losses and adaptive losses related to habitual diet occur at constant rates during the postabsorptive and postprandial phases of the cycle, so that meal-related inefficiency is the increased rate of loss during feeding. Feeding involved frequent small meals to maintain a metabolic steady state. Measurements were made in normal adults adapted (2 weeks) to protein intakes of 0.36, 0.77, 1.61, and 2.12 g/kg. After an overnight fast a primed 8-hour intravenous $[^{13}\text{C}]$-l leucine infusion (4 h fasting, 4 h feeding) was employed, with meals given every 30 min at intakes equal to $\frac{1}{12}$ (daily intake/h). Thus leucine and predicted N balance can be measured in postabsorptive and postprandial states. PPU is then calculated from $\frac{\Delta N_{\text{balance}}}{\Delta N_{\text{intake}}}$, in turn calculated from $(\Delta \text{leucine}_{\text{balance}}/\Delta \text{leucine}_{\text{intake}})$ over the fast/feed transition.

These studies helped identify the adaptive component of the metabolic demand, which increased with intake as shown in Table 1. Calculation of PPU from leucine balance indicated remarkably constant values over this wide range of intakes, with a mean value of 0.78 ± 0.21 [6]. However, as shown in Figure 3 calculation of the true PPU based on nitrogen balance indicates near perfect utilization at all meal concentrations (0.97 ± 0.23). Thus it is clear that in subjects adapted to varying protein concentrations, protein utilization does not vary with protein concentration as measured in this way. The fact that a similar proportion of the intake was utilized at each dietary protein level reflected the increasing amplitude of diurnal gains and losses with the increasing habitual protein intake. Table 1 also shows that the calculated apparent protein requirement increased with intake because of the increasing metabolic demand. What these studies do not show is whether the protein concentration influences PPU in subjects not adapted to the varying intakes. It might be predicted, for example, that if there is a fixed
Postprandial Protein Utilization: Implications for Clinical Nutrition

A

Leucine oxidation

Fasting

Meals

Meal-related losses

Habitual protein intake-related losses

Obligatory losses

$[^{13}\text{C}]-\text{leucine}$ Infusion balance

Time (h)

B

Leucine oxidation

Fasting

Meals

Low protein

High protein

Meal-related losses

Habitual protein intake-related losses

Obligatory losses

$[^{13}\text{C}]-\text{leucine}$ Infusion balance

Time (h)

C

Leucine oxidation

Fasting

Single meal

Meal-related losses

Habitual protein intake-related losses

Obligatory losses

$[^{13}\text{C}]-\text{leucine}$ Infusion balance

Time (min)
upper limit to the lean body mass – as suggested within the protein-stat model of muscle mass regulation [19] – subjects habituated to a low protein intake with a consequent low metabolic demand and low postabsorptive losses may not be able to deposit all of the dietary protein from high protein meals. We have shown that in subjects habituated to a high protein diet (1.9 g/kg-day), within the first 14 days of a change to a lower intake of 0.8 g/kg-day the PPU was decreased, owing to a lack of adaptation of oxidative losses, especially in the postprandial state [17].

**Effects of Age on Postprandial Protein Utilization**

The elderly represent an obvious population group of interest in the current context, as it has always been an assumption that protein utilization may be problem for old people – for example, ‘... it is an accepted fact that protein utilization is less efficient in the elderly’ [20]. In fact we know relatively little about the extent and regulation of PPU in the elderly or at any other age. We recently attempted to examine this in a modified leucine balance protocol which allows measure of PPU in a slightly different way.

In this protocol (Fig. 2B) PPU is determined during a small frequent-meal steady-state protocol [7, 8, 21]. This includes three 3-hour balance periods: the postabsorptive state and sequential feeding of two different levels of dietary protein. We fed frequent small liquid milk meals of either 2% protein energy (low protein, LP) or approximately 14% protein energy (high protein, HP) with constant carbohydrate intakes. The actual intake during the HP period was matched to the habitual intake of the subjects, who were 25 normally mobile adults aged 20–90 years [8]. Sequential feeding of LP and HP meals is an attractive protocol as it allows study of the kinetic responses to energy

**Fig. 2.** Leucine balance protocols for the evaluation of postprandial protein utilization (PPU). Leucine balance is measured as leucine intake minus leucine oxidation, and PPU is calculated from these data after transforming leucine balance into nitrogen balance (3.93 µmol leucine/mg N) as follows:

- **Frequent small meal fast feed protocol**

  \[
  \text{PPU} = \frac{\Delta N_{\text{balance}}(\text{fast feed})}{\Delta N_{\text{intake}}(\text{fast feed})} \approx \frac{\Delta \text{leucine}_{\text{balance}}(\text{fast feed})}{\Delta \text{leucine}_{\text{intake}}(\text{fast feed})};
  \]

- **Frequent small meal low protein/high protein protocol**

  \[
  \text{PPU} = \frac{\Delta N_{\text{balance}}(\text{HP} - \text{LP})}{\Delta N_{\text{intake}}(\text{HP} - \text{LP})} \approx \frac{\Delta \text{leucine}_{\text{balance}}(\text{HP} - \text{LP})}{\Delta \text{leucine}_{\text{intake}}(\text{HP} - \text{LP})};
  \]

- **Single large meal fast feed protocol**

  \[
  \text{PPU} = \frac{N_{\text{utilization}}}{N_{\text{intake}}} \approx \frac{\text{leucine}_{\text{intake}} - \text{cumulative meal-related leucine losses}}{\text{leucine}_{\text{intake}}};
  \]
### Table 1. Factors influencing postprandial protein utilization

#### Protein intake

<table>
<thead>
<tr>
<th>Protein intake</th>
<th>Intake</th>
<th>Metabolic demand</th>
<th>PPU</th>
<th>Apparent requirement</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mean</td>
<td>SD</td>
<td>mean</td>
<td>SD</td>
</tr>
<tr>
<td>Protein intake</td>
<td>g protein/kg-day</td>
<td>g protein/kg-day</td>
<td>g protein/kg-day</td>
<td>g protein/kg-day</td>
</tr>
<tr>
<td>0.40</td>
<td>0.03</td>
<td>0.43</td>
<td>0.08</td>
<td>1.03</td>
</tr>
<tr>
<td>0.77</td>
<td>0.04</td>
<td>0.57</td>
<td>0.10</td>
<td>0.92</td>
</tr>
<tr>
<td>1.51</td>
<td>0.08</td>
<td>0.85</td>
<td>0.12</td>
<td>0.94</td>
</tr>
<tr>
<td>2.00</td>
<td>0.13</td>
<td>1.06</td>
<td>0.22</td>
<td>1.00</td>
</tr>
</tbody>
</table>

#### Age and sex effects

<table>
<thead>
<tr>
<th>Age</th>
<th>Sex</th>
<th>Metabolic demand</th>
<th>PPU</th>
<th>Apparent requirement</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mean</td>
<td>SD</td>
<td>mean</td>
<td>SD</td>
</tr>
<tr>
<td>Young adult</td>
<td>F</td>
<td>0.83</td>
<td>0.14</td>
<td>0.99</td>
</tr>
<tr>
<td>Young adult</td>
<td>M</td>
<td>0.90</td>
<td>0.06</td>
<td>1.05</td>
</tr>
<tr>
<td>Middle age</td>
<td>M</td>
<td>0.87</td>
<td>0.18</td>
<td>1.01</td>
</tr>
<tr>
<td>elderly</td>
<td>F</td>
<td>0.52</td>
<td>0.14</td>
<td>0.92</td>
</tr>
<tr>
<td>elderly</td>
<td>M</td>
<td>0.58</td>
<td>0.17</td>
<td>1.05</td>
</tr>
<tr>
<td>All</td>
<td>M</td>
<td>0.74</td>
<td>0.21</td>
<td>1.00</td>
</tr>
</tbody>
</table>

#### Protein quality and meal size

<table>
<thead>
<tr>
<th>Protein quality and meal size</th>
<th>Metabolic demand</th>
<th>PPU</th>
<th>Apparent requirement</th>
<th>Estimated average requirement</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Protein</td>
<td>Lysine</td>
<td>Protein</td>
<td>Lysine</td>
</tr>
<tr>
<td></td>
<td>g protein/kg-day</td>
<td>g protein/kg-day</td>
<td>g protein/kg-day</td>
<td>g protein/kg-day</td>
</tr>
<tr>
<td></td>
<td>mean</td>
<td>SD</td>
<td>mean</td>
<td>SD</td>
</tr>
<tr>
<td>Small meals</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Milk</td>
<td>0.73</td>
<td>0.18</td>
<td>1.00</td>
<td>0.09</td>
</tr>
<tr>
<td>Wheat</td>
<td>0.73</td>
<td>0.18</td>
<td>0.68</td>
<td>0.06</td>
</tr>
<tr>
<td>Large meals</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Milk</td>
<td>0.83</td>
<td>0.23</td>
<td>0.99</td>
<td>0.04</td>
</tr>
<tr>
<td>Wheat</td>
<td>0.84</td>
<td>0.15</td>
<td>0.59</td>
<td>0.05</td>
</tr>
</tbody>
</table>

*p: small vs. large 0.058 0.032 0.052 0.052*

---

1. Normal adults: recalculated from Price et al. [14].
2. Recalculated from Fereday et al. [7, 8].
3. Millward et al. [25].
4. Fereday et al. [32, 33].
Fig. 3. Influence of meal protein concentration on the efficiency of postprandial protein utilization. Measurements made in the fast feed protocol as shown as Figure 2A.

(mainly insulin) and to protein (insulin plus amino acids) during the two feeding periods [21]). In this case there are two options for calculating PPU – from balance changes between the two protein intakes or between postabsorptive and HP intake (in effect the same value as obtained in the fast/feed protocol described above). The first calculation examines PPU only in relation to the protein, while the second includes influences of all aspects of the meal (that is, including the energy effects). In fact as most of the changes in balance are between the LP and HP intakes, the differences between these two values are not marked. In theory calculation of PPU from the LP/HP transition has the advantage of allowing the N equivalence of leucine balance to be calculated with more certainty. While an estimate of the leucine to N ratio of tissue protein can be made (assuming mixed tissue proteins are deposited), the leucine to N ratio of the oxidative loss component is unknown. Thus theoretically, the change in leucine balance between two balance points in the zero and positive balance range should allow calculation of a more accurate estimate of PPU, and these values are presented here.

It is clear from the data in Table 1 and Figure 4 that PPU is not influenced by age, at least in this healthy population, and that on average in this protocol milk protein was perfectly utilized. The only identifiable age-dependent variable is the lower metabolic demand and consequent lower apparent protein requirement in the elderly (7). This elderly group was mobile and well nourished so it remains to be seen how immobile or less well nourished elderly people handle protein.
Effects of Meal Protein Quality on Postprandial Protein Utilization

The ability of wheat and other plant protein sources to support nitrogen balance in humans has been studied, but the variability in such trials results in great uncertainty about the overall importance of protein quality in human nutrition [22]. A diurnal cycling model of nitrogen homeostasis has important implications for indispensable amino acid (IAA) requirements and consequently for the issue of protein quality. With substantial postprandial protein deposition there will be an obvious need for dietary IAA which will not differ markedly from the need for growth. However, the influence of variation in dietary protein quality on postprandial protein utilization and balance is complex [4, 16].

As reviewed elsewhere [16], there is evidence that the amplitude of diurnal cycling and the need for postprandial repletion varies with protein quality, as shown by the studies of Marchini et al. [23] with amino acid mixtures containing varying amounts of essential amino acids. Postabsorptive leucine balance, and hence postprandial gain needed for repletion, varied by 60% according to the amounts of the IAA in the mixture. Also, IAA needs for postprandial repletion may differ from the needs for growth because of the possibility of recycling of amino acids from postabsorptive catabolism into net deposition during feeding. This is likely to be the case for lysine and threonine: their oxidation is less regulated, they have a substantially larger free pool size than the branched chain aromatic and sulfur amino acids, and they are less rapidly cleared from the free pool after a meal [24]. Recycling of these amino acids could provide a substantial...
part of their needs for postprandial deposition according to the extent of the habitual protein intake if the diet was deficient. However, our ability to predict from first principles how dietary deficiencies of IAA influence the efficiency of protein utilization is poor, on the basis of our current understanding of how postprandial oxidation within the adaptive component of the metabolic demand is regulated (Fig. 1). This substantial component of the metabolic demand represents the combined oxidation of a mixture of amino acids, the composition of which will be determined in a complex way by the kinetic characteristics of the catabolic pathway of each amino acid and its free pool size. This in turn will be set by the balance between dietary supply and net protein accretion. While a reduction in the oxidation rate of an individual limiting IAA like lysine would allow balance to be maintained, not enough is known about the kinetic characteristics of amino acid oxidation \textit{in vivo} to predict the effects which individual amino acids are likely to have on PPU.

We have investigated the extent of adaptation to an acute change of dietary protein from an animal protein source to wheat protein using two protocols. In the first [25], we have used the small frequent meal $^{13}$C balance protocol as in Figure 2B in a group of healthy adults. The protein source was stone-ground wholemeal bread fed with margarine and potato starch, and with fit providing 30 and 18% of the energy in phases ii and iii, respectively. As shown in Figure 5 and Table 1, PPU calculated from the change in balance between the LP and HP periods was 1.00 ± 0.09 and 0.68 ± 0.06 for milk and wheat, respectively. These data show the importance of calculating PPU on the basis of nitrogen balance as opposed to uncorrected leucine balance as discussed above, as the values obtained from the latter indicate similar utilization efficiencies from each protein source (0.85 ± 0.05 for wheat and 0.81 ± 0.07 for milk). Only when their compositional differences are taken into account (the leucine content of milk is higher than tissue protein, and that for wheat is lower) are the true values indicated.

As indicated above, it is not possible to predict completely from first principles what the efficiency of wheat utilization should be, but it is possible to make an estimate with no assumptions about adaptive responses. The way this is done is to assume that wheat utilization is limited only by lysine content, and to calculate the ratio of the lysine content of the wheat fed to that of the measured lysine deposited from the milk protein (from net leucine deposition convened to tissue protein gain and the lysine content of tissue protein). When this is done, a PPU of 0.68 is indicated, higher than the value predicted for wheat protein utilization (0.26 ± 0.02) in these subjects under the conditions of the experimental protocol. Potential explanations of this are adaptive responses to the lysine deficiency of the meal by (i) recycling free lysine into protein, and (ii) reducing lysine oxidation. Whatever the explanation, it is clear that wheat protein is utilized more efficiently than would be predicted.
Effects of Meal Size on Postprandial Protein Utilization

One important practical issue of clinical importance relates to whether there are advantages of feeding frequent small meals as opposed to less frequent large meals. This is often referred to as grazing or gorging, at least in the context of energy intakes and balance. On the basis of the results in Figure 3, meal protein content has little influence on PPU, although, as discussed above, these results were obtained in subjects adapted to the varying protein intakes. This means that in each case the increasing protein intake was fed to subjects with increasing postabsorptive losses and therefore an increasing metabolic demand. The interesting question is whether a pattern of feeding can be devised that results in a lower metabolic demand (that is, smaller postabsorptive losses) for a given overall rate of protein intake. In this case more of the dietary protein would be available for net deposition, and improved overall nitrogen balance would follow.

In fact the few data relating to this involve conflicting results. Nitrogen balance was higher in young women fed three equal daily protein meals compared with three meals of which only two contained protein [26]. However, similar studies in young men failed to identify any differences [27]. More recently Arnal et al. [28] have investigated this in an elderly population in whom they compared the effect of either ‘pulse’ feeding, providing 80% of the daily protein intake in the mid-day meal and the remaining 20% in the morning and evening meals, or spreading the protein intake over four meals. They measured nitrogen balance and changes in the lean body mass in elderly...
women after first reducing their protein intakes to 0.74 g/kg-day for 14 days and then feeding 1.05 g/kg-day by either pulse or spread feeding regimens. They found a more positive N balance in the pulse than in the spread group, indicating a more efficient protein retention with the larger protein meals. How these results were achieved is not clear as the kinetic measurements deployed, based on $^{15}$N glycine-end product studies, do not allow assessment of the extent of postabsorptive and postprandial losses.

We have examined the response to a single 50 g/500 kcal meal of either milk or wheat given 3 h after the start of a [13C-$\text{-l}$]-leucine infusion, with leucine oxidation measured from frequent sampling over the following 6 h (Fig. 2C) [5, 6]. We assumed that bicarbonate recovery increased from 0.76 (postabsorptive) to 0.911 (postprandial) in parallel with the increase in CO$_2$ production. The meal, 0.5 g protein and 22.9 kJ/kg, was either fresh skimmed milk with dissolved potato dextrose or a dry fried pancake made of equal portions of wheat gluten and plain flour, and was designed to give 50% of the subject’s daily intake with a relatively high protein to energy value (30% protein energy). The wheat pancake was accompanied by a drink containing dissolved hydrolyzed potato dextrose and paracetamol (1.5 g added as an absorption marker), and was consumed over 15 min, 180 min after the start of the leucine infusion. The absorption of the meal was monitored by measurements of plasma glucose, insulin, leucine, and paracetamol.

In this case, given that the subjects were not in steady-state conditions, leucine balance was calculated from leucine intake minus the cumulative meal protein-related increase in leucine oxidation – that is, postmeal oxidation minus premeal postabsorptive oxidation level. Measurements were made over 6 h after the meal, during which time leucine oxidation returned to baseline, indicating complete utilization.

As shown in Figure 6, the main differences between the two meals were related to the time course of postprandial events. Thus leucine oxidation peaked earlier after the milk meal than after the wheat meal. The cumulative increase in oxidation was $20.2 \pm 3.1$ and $26.6 \pm 8.5\%$ of the intake for milk and wheat, respectively – that is, equivalent to PPU values based on uncorrected leucine balances of 0.80 and 0.733 for milk and wheat. When calculated as nitrogen balance, these values are equivalent to 0.98 ± 0.05 and 0.59 ± 0.05. As shown in Figure 5, the value for wheat is a little lower than that observed with the small meals, but the significance of the difference is marginal ($p = 0.06$). Clearly more measurements would be needed to establish whether this is a true meal size-related difference.

As discussed above, assuming that it is limited only by lysine content, a predicted value for wheat protein utilization can be calculated in these subjects under the conditions of the experimental protocol as the ratio of the lysine content of the wheat fed to that of the measured lysine deposited from the milk protein. The predicted value for wheat protein utilization in these subjects under the conditions of the experimental protocol was 0.29 ± 0.01, similar to the value
Fig. 6. Cumulative increased leucine oxidation after milk and wheat meals expressed as a fraction of leucine intake. Leucine oxidation is calculated from the postmeal rate less the premeal value (as in Fig. 2C).

obtained with the steady-state multiple small meal protocol and much lower than the observed value.

These results tell us two things about PPU. First it appears that as far as the experimental protocols we have used are concerned the overall efficiency of protein utilization after a large meal does not differ markedly from that after frequent small meals, certainly for milk, with near perfect utilization in each case, and with only a small (13%) and marginally significant difference for wheat (Fig. 5). Second, as far as wheat versus milk is concerned, while PPU for wheat appears to be 30–40% lower than for milk, it is much higher than would be predicted. Thus, as we have suggested previously, the pool size of lysine and its less actively regulated oxidation rate (compared with leucine for example) allows lysine in the free pool to supplement the limited dietary lysine in wheat, thereby improving its utilization.

On the basis of these data it is possible to calculate a requirement for lysine. This involves calculation of the estimated average protein requirement for wheat derived from the average requirement for high quality protein (0.6 g/kg) and the PPU (that is, 0.6/PPU), and the lysine content of that amount of wheat. As shown in Table 1, with the small meal protocol the value was 0.89 ± 0.08 g protein/kg-day and 23.2 ± 2.0 mg lysine/kg-day, while with the large meal protocol the values were 1.03 ± 0.09 g protein/kg-day and 26.7 ± 2.3 mg lysine/kg-day. These values are somewhat higher than those derived from the nitrogen balance studies of Jones et al. [29] which, after adjustment for miscellaneous losses and recalculation on a body weight basis [30], indicate a lysine
requirement of 19 mg/kg-day. However, as mentioned above, within our adaptive model of protein homeostasis the metabolic demand for lysine will fall in response either to a lower protein intake (with reductions in both the postabsorptive losses and postprandial gains as we have reported) [14], or to a lower quality of protein intake (as indicated by the leucine balance data of Marchini et al. [16, 23]. Thus we would expect our subjects habituated to a diet supplying 1.2 g protein and 74 mg lysine daily to have a larger lysine requirement and therefore a lower efficiency of utilization than in the studies of Jones et al. [29], in which some adaptation to the lower intakes would have occurred.

Factors Influencing Leucine Oxidation and Protein Utilization

Predicting the likely response to variation in meal size from first principles is difficult. Thus the three processes which mediate net protein deposition – stimulation of oxidation, inhibition of proteolysis, and stimulation of protein synthesis – are each sensitive to amino acid supply and to a more variable extent to insulin [5, 9, 21]. This means that the net influence of the systemic entry of a large amount of amino acids will be a balance between the potential increase in intracellular amino acid levels resulting in increased rates of oxidation and protein synthesis, and a more marked inhibition of proteolysis limiting any increase in intracellular amino acid levels.

As discussed previously [5, 6], in these large meal, non-steady-state studies the comparison of the time course of changes in leucine oxidation vs. plasma concentrations of leucine, MC, and insulin does afford some insight into the regulatory mechanisms of leucine oxidation. It would appear that while the time course of postprandial changes in leucine oxidation very approximately reflects plasma leucine concentrations, it is by no means a simple relation, with evidence that leucine oxidation is activated by elevated insulin levels and is not simply substrate driven. On the other hand in circumstances where insulin increases in advance of any increase in leucine concentration, such as after the wheat meals described here, leucine oxidation is not increased, suggesting that in the absence of increases in leucine concentration, leucine oxidation cannot be markedly stimulated by insulin.

As far as protein synthesis and proteolysis are concerned, we have recently investigated how the response of these variables to feeding regulates PPU [9]. We showed that the key response is an inhibition of proteolysis (by separate and additive receptor-mediated influences of amino acids and insulin), sufficient to limit any increases in amino acid levels and consequent oxidation. It is likely that protein synthesis is regulated by intracellular amino acid levels; however, postprandial stimulation through increases in amino acid levels appears to be unhelpful because when it occurs there are parallel increases in amino acid oxidation and less efficient protein utilization. As PPU is by definition inversely correlated with the increase in oxidation, stimulation of protein synthesis is
Postprandial Protein Utilization: Implications for Clinical Nutrition

negatively correlated with PPU. Thus subjects who show maximal inhibition of proteolysis on feeding have minimal postprandial increases in tissue free amino acid concentrations, thereby limiting increases in amino acid oxidation and, coincidentally, protein synthesis. The calculated amino acid sensitivity of proteolysis (% inhibition/incremental amino acid intake) predicted PPU \( r = 0.76 \). Thus in this series of 25 adults of all ages ranked according to PPU, the top quintile achieved their postprandial deposition by maximal inhibition of proteolysis by protein feeding, with minimal increases in free amino acid concentrations, oxidation, and protein synthesis. The lowest quintile used a mechanism whereby a lesser inhibition of proteolysis was combined with a greater stimulation of protein synthesis, but with more oxidation and hence lower efficiency. This indicates that the efficiency of protein utilization in individuals, and a component of their apparent protein requirement is determined by the sensitivity to amino acid supply of insulin-mediated inhibition of proteolysis, and this varied by a factor of 5 between the top and bottom quintiles of PPU. The molecular nature of this variability is unknown. It may well be that in hospital patients the persistent catabolic state associated with an inflammatory state, which is known to involve inhibition of insulin action mediated by tumor necrosis factor and possibly other cytokines, also involves reduced sensitivity to amino acids of the insulin-mediated inhibition of proteolysis that occurs with feeding. However, this has yet to be established.

Conclusions

These \(^{13}\)C-leucine balance results suggest that when PPU is measured in the way described here it is shown to occur in normal adults with a high efficiency, is only marginally influenced by meal size, and the effects of protein quality are less than would be expected. This may reflect the capacity to recycle lysine liberated in the postabsorptive state into postprandial deposition.

These measurements of PPU have clear potential for monitoring the efficacy of nutritional support in various clinical situations such as in postoperative hospital patients and in elderly patients at home who are immobile or more obviously wasted than the subjects we have studied. Understanding overall nitrogen balance in patients at risk requires study throughout the diurnal cycle and this must be related to the physiological state of the subjects and the nature of the food consumed. The methods described here allow such study.

Acknowledgments

I am grateful for the support of Research into Aging, the Nestlé Foundation, and the Medical Research Council of Great Britain.
References

Discussion

Dr. Reeds: Does \( \Delta \) leucine intake refer to your estimate of the postabsorptive leucine loss plus the leucine that you receive in the diet or is it actually the leucine intake?

Dr. Millward: ‘\( \Delta \)’ in that equation relates to the change in intake from the low protein to the high protein phase, and so nitrogen utilization under those circumstances is leucine utilization, which is the change in leucine intake minus the change in the increased leucine oxidation above the postabsorptive level.

Dr. Reeds: Also, do you assume, in calculating it, that the postabsorptive oxidative losses are continuing, so that your meal-related losses are a genuine increment above that?

Dr. Millward: Yes, we actually measured that.

Dr. Reeds: But you are assuming that those are continuing without the meal affecting them.

Dr. Millward: Clearly the model allows for better than 100% utilization.

Dr. Reeds: That’s what’s worrying me.

Dr. Millward: Well, one does observe that, of course, when one uses the fasted to fed transition, because we know — and we’ve done this in our studies — that some individuals show a fall in leucine oxidation in response to energy intake, and we know that if you give a dose of energy you stimulate insulin secretion, you lower amino acid levels, and you can lower oxidation. So it’s possible to get infinitely large protein utilization by getting an improved utilization with zero intake in this model. That’s why we’ve shifted to using the low protein to high protein transition, because we then don’t see the energy effect. We’re doing the studies at a constant insulin level, at a constant energy intake, and we’re
really moving from subjects in balance to subjects in positive balance. The oxidative losses in the postabsorptive state don’t enter the equation under those circumstances.

Dr. Lundholm: I was very impressed by your skill in performing these kinds of feeding studies with isotope infusions. When I have tried to do similar studies, the reviewers have always asked what evidence I had for steady-state conditions – and my arguments always seem to be imperfect! I find it very difficult to get the scientific community to accept these kinds of studies, where we infuse isotopes over short periods and then manipulate the conditions by feeds or infusions and so on. Could you comment on the steady-state aspect, because I think that’s very critical before we can interpret the data?

Dr. Millward: Frequent small meals, which involve half-hourly feeding, do appear to achieve a steady state as judged by no significant change in plasma leucine or α-ketoisocaproate (KIC) concentrations during each feeding phase, and a constant level of KIC enrichment. Clearly with the single large meals – which we haven’t published yet and about which I’m expecting exactly the same sort of harassment from the reviewers – there are non-steady-state conditions, and we use non-steady-state kinetics to analyze them. We’ve got all the data on leucine appearance and non-oxidative disappearance calculated using non-steady-state kinetics, where you have to make assumptions of distribution spaces. It’s a bit of a nightmare. Fortunately, the leucine balance data are very simple. There are no kinetic problems. One has CO₂ production rate at a fixed point in time and you have a KIC enrichment at a fixed point in time. You don’t have to do any fancy calculations relating to the non-steady-state in order to measure leucine balance, but you do if you want to measure proteolysis and protein synthesis. What I’m concerned about is the thing that we have never addressed traditionally in these tracer studies: the tracer studies were developed to measure turnover, and we’ve never really paid proper attention to balance; for example, the ¹⁵N-glycine method – which is a very useful method for getting a global picture of protein turnover – is actually terribly difficult to interpret in terms of protein synthesis and breakdown, simply because the balance part of that is nitrogen excretion over the course of the measurement, and that is very difficult to determine. So I think if you’re careful, and state what it is that you’re examining, then you can satisfy reviewers that their concerns are not warranted.

Dr. Young: Your relative PPU values between wheat and milk are very satisfying as far as I’m concerned; they are consistent with our earlier nitrogen balance studies where we compared the net protein utilization of wheat and meat. But your actual values for PPU (that is, 1 for milk) are considerably higher than the NPU values for milk. Why the difference, in a quantitative sense?

Dr. Millward: This has puzzled me but there is a simple explanation for it. When we do the NPU assay in humans, in effect we do nitrogen balance studies in the submaintenance range. So we give people increasing levels of intake of the protein under investigation, and we measure nitrogen balance. When you do that for egg protein or milk protein, or beef, or whatever, you get an NPU value considerably less than 1. There are some studies with egg where you get a value of 0.5. With my model of protein requirements, which says that the requirement increases with an increased protein intake, the actual amount that is utilized (Fig. 7) is ΔB₂ not ΔB₁, so that explains why the human data give a value considerably less than 1. When you do the assay in the rat, you do it in growing animals and most of the utilization is for growth. Also we know that rats use proteins nearly perfectly, because the adaptive component, if it exists at all in the rat, is very small, so ΔB₂ is nearly the same as ΔB₁. I think this is the explanation.

Dr. Beaufrère: You showed us some data on your estimates for protein requirements in elderly people and they are 20 or 30% lower than in young adults. As you know, there is a tendency to suggest that elderly people have an increased requirement, from nitrogen balance data for example. Could you comment on that?
Postprandial Protein Utilization: Implications for Clinical Nutrition

Dr. Millward: We should ask how applicable these laboratory measurements are to a free living situation, and I don’t know the answer to that. We are attempting to probe basic physiology, to determine whether there is anything wrong with elderly people in terms of their ability to use protein under these kinds of conditions, or any difference in their metabolic demands. I can’t think of any particular reason why our data should be incorrect. On the wider issue of whether elderly people need more protein, I reviewed this issue in the American Journal of Clinical Nutrition [1], where I made the point that there is absolutely no evidence that differences in apparent nutritional status – in terms of albumin or mid-upper arm circumference or any other such measures – are actually related to protein intake. In fact, the data of Munro et al. [2], from the biggest single study that’s ever been done, actually showed an inverse relation between nutritional status and protein intake in their cohort of elderly east coast North Americans. And if you look at the data from Southampton [3] on the relation between nitrogen balance and protein intake in elderly people in institutions or free living, you see that while those in institutions have a lower nitrogen balance than those who are free living, there is no relation at all with protein intake. So I know of no evidence to suggest that varying protein intake within the normal range is detrimental for elderly people.

Dr. Lundholm: Maybe I misunderstood something, but you showed a slide where you indicated that protein utilization was the same when protein intake was at the 5% level as it was at the 20% level, but the difference between the 5% level and the 20% level was that the variation within the group was larger at 5% protein intake than at 20%. I have difficulty in interpreting this, because those data appear to suggest that even at the very low protein intake, you can take up and utilize proteins as efficiently as you can at the 20% level, and that would fit with Hamish Munro’s data and the other Southampton data you referred to, but it doesn’t make sense to me immediately. Have I missed something?

Dr. Millward: It only barely makes sense to me, and I’ve been looking at these data for a long time! The point is that when one is looking at the transition from the postabsorptive stage to the fed state and feeding very small amounts of protein, the changes in leucine oxidation are very small and can go in either direction, so one has a very big error term. Thus it’s not a very precise method and variability is a methodological problem, but the mean values are the same as on a high intake. My measurements relate to individuals.
who had been habituated over the previous fortnight to those diets. Thus the people fed
the high protein diet had been eating a high protein diet for the previous two weeks and
the same for the low protein diet. What we haven’t done is to take individuals on the
same intake and do that particular study. I’m not sure what the answer would be.

Dr. Lundholm: So it may be a matter of adaptation.

Dr. Millward: Yes.

Dr. Reeds: When you do an overnight fast of, let’s say, 14 h and you’ve spent the
last 4 weeks on a very high protein intake, isn’t there a real danger that you’re actually
in a different postabsorptive state? It may appear that you’ve got a higher need because
you haven’t actually reached basal by that point.

Dr. Millward: Dr. Young has adopted his 24-hour protocol precisely to get over these
sorts of problems, but the rest of us have to be content with doing these rather cut down
experiments the best we can. Now if I had more support, of course, I could do much
better studies!

Dr. Lundholm: Could you draw some final conclusions about the clinical nutritional
point of this.

Dr. Millward: The main practical point is that we can be misled to a certain extent
about exactly what it is we have to measure. When one approaches this kind of model
recognizing that one has to consider two components, the metabolic demand and the
efficiency of utilization, it becomes clearer. It’s possible, for example, that when you
give a pulse diet you may lower the adaptive component of metabolic needs rather than
change the efficiency of utilization. In patients it’s exactly the same issue. We need to
know how much they actually need. What is their metabolic demand? That’s the baseline
for starting to feed them. And then we need to know what the efficiency of utilization
is. We’ve done an awful lot of work over the last 30 years measuring protein synthesis
and protein breakdown and arguing over whether one goes up or the other goes down,
but what really matters is how much they need and how efficiently the feed is utilized.

References

1. Millward DJ, Fereday A, Gibson NR, Pacy PJ. Ageing, protein requirements and protein turn-
2. Munro HN, McGandy RB, Harz SC, Russell RM, Jacob RA, Otradovec MA. Protein nutriture