Dietary Protein and Nitrogen Utilization

Daniel Tomé and Cécile Bos

ABSTRACT The first approach used to study the utilization of nitrogen in the body was based on the measurement of nitrogen balance. Limitations to this method reside in the difficulty of precisely determining nitrogen losses and, more specifically, miscellaneous N losses. These shortcomings are particularly restrictive when investigating adaptation of the organism to different levels of protein intake. The principal issue is to gain a better understanding of the adaptive processes that occur with high protein intakes and the possibility of producing a net protein accretion by nutritional means in different situations. The investigation of protein metabolism in relation to dietary proteins, with a focus on the postprandial phase of nitrogen diurnal cycling, enables a clearer determination of the metabolic pathways for dietary nitrogen as a function of different factors, which include the habitual protein level and intrinsic protein characteristics. We propose that this in vivo approach in humans should be used to validate simpler indices of the nutritional value of proteins.

The nutritional value of dietary proteins in humans depends on their ability to meet nitrogen and amino acid requirements for growth and maintenance, but the level and type of these requirements remain unclear in humans (Millward and Pacy 1995, Munro 1969, Rennie et al. 1994, Young and Pellett 1988). Levels of body protein remain relatively constant throughout adult life, despite qualitative and quantitative variations in dietary protein intake. High protein diets provide as much as ~180–200 g/d in adults, whereas the minimum intake is currently evaluated at 50 g/d. Under these conditions, reaching a nitrogen balance involves adaptations that may be associated with adverse metabolic consequences (Young 1986, Young et al. 1987). It is well accepted that the nutritional value of proteins may differ substantially. Variable factors include their essential amino acid content and digestibility. Based on the different methods used to assess dietary protein quality in humans, the protein digestibility–corrected amino acid score (PDCAAS) takes account of these two parameters with respect to amino acid requirements (FAO/WHO, 1990). However, the definition of protein and amino acid requirements is complex and makes it difficult to assess the nutritional value of proteins by using scores as a means of predicting net protein utilization.

Nitrogen balance and nitrogen requirements

Nitrogen requirements include what is needed for both tissue protein synthesis and the production of several nitrogenous compounds involved in a range of functions (hormones, neurotransmitters, immune competence and peroxidative defenses). Under specific physiological and dietary conditions, the dietary requirements for protein, amino acid and nitrogen are determined by the nature of the metabolic demand that must be satisfied. An evaluation of protein quality must therefore take into account the different processes involved in amino acid and nitrogen homeostasis. The achievement of nitrogen homeostasis involves a complex series of changes in rates of whole body protein turnover, amino acid oxidation, urea production and nitrogen excretion during the fasting, fed, postprandial and postabsorptive periods of the day.

Human nitrogen requirements are usually determined from the nitrogen balance. The usual procedure is to regress nitrogen balance on intake and to define the requirement as the intake level that would produce a zero balance, i.e., equality of dietary N intake and N losses (Fig. 1). Nitrogen losses occur in different ways. They mainly arise from urinary losses in the form of urea, ammonia and creatinine but also in the form of fecal and miscellaneous losses (Calloway and Margen 1971) (Table 1). Minimum nitrogen losses (“obligatory nitrogen losses” (ONL)] were measured in subjects fed a protein-free diet for 1 week. Under these conditions, nitrogen losses were estimated at 36 mg/kg/d in urine, 12 mg/kg/d in feces and 8 mg/kg/d in miscellaneous nitrogen losses (sweat, sebum, des-
quamations, nails, hairs and saliva) (Manatt and Garcia 1992). Obligatory oxidative losses and indispensable ileal amino acid losses have been reported to reach 162 and 18 mg/kg/d, respectively (Fuller et al. 1994, Young et al. 1989) (Table 2). We determined total ileal nitrogen losses as reaching 9 mg/kg/d, i.e., 16% of ONL. Indispensable ileal amino acid losses represent 10% of obligatory oxidative losses. From these estimations, ONL represent 54 mg/kg/d and correspond to a protein requirement level of 0.34 g/kg/d (FAO/WHO, 1985). Because dietary protein utilization does not achieve 100% efficiency, it has been suggested that an intake of 0.6 g/kg/d of well-balanced protein will achieve a zero nitrogen balance. The adequacy of this diet has been reported in studies conducted over 2- or 3-mo periods (FAO/WHO 1985). A safety coefficient is added to this figure so that the final recommendation for dietary protein is 0.75 g/kg/d. In children, the requirements for growth must be integrated in addition to maintenance requirements.

It is important to emphasize that these values represent the minimum recommended protein intake. Studies investigating the metabolic response to different protein intakes have considered the ability of the body to make metabolic adjustments to a wide range of protein intakes (0.75–2 g/kg/d). In addition, there are various limitations to determining the nitrogen balance, and as recently pointed out (Rand and Young 1999): “Nitrogen balance estimates are highly dependent on the assumed amount of N miscellaneous losses... further studies on these losses and on the factors that influence them are essential.” First, there is a slight difference between large values for N intake and N losses. Second, it is well recognized that the nitrogen balance technique overestimates N intake and underestimates N losses. This is mainly due to the difficulty in assessing N gas losses after denitrification by the colonic microflora, N losses through the skin (urea) and expired air (ammonia) and the nitrate content in food and urine, which is not measured using the Kjeldahl method.

Increases in protein intake and nitrogen balance

People often consume more protein than the theoretical requirement based on nitrogen balance estimations. The effect of an increased protein intake on the whole body nitrogen balance and protein turnover must be determined. In particular, it is very important to elucidate the consequences of increasing the nitrogen intake with regards to different nitrogen pathways. The type of protein and nitrogen pools likely to be modified by the level of nitrogen intake also must be clarified. This leads to the question of the optimum protein intake and the possibility that this optimum level may be higher than current recommendations (Millward, 1999).

Increasing the protein intake induces a series of adaptive processes (Fig. 2). The most conspicuous adjustment is an increase in amino acid oxidation and in subsequent nitrogen excretion, mainly as urea and especially pronounced in the fed state. There is a trend toward an increase in the nitrogen

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**TABLE 1**

<table>
<thead>
<tr>
<th>Body localization</th>
<th>Nature of N ion</th>
<th>Quantity</th>
<th>mg N/d</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine</td>
<td>Urea, ammonia, creatinine</td>
<td>10,000</td>
<td>84.4</td>
<td></td>
</tr>
<tr>
<td>Feces</td>
<td>Diet residue, endogenous</td>
<td>1500</td>
<td>12.7</td>
<td></td>
</tr>
<tr>
<td>Dermal</td>
<td>Skin, sweat</td>
<td>200</td>
<td>1.70</td>
<td></td>
</tr>
<tr>
<td>Hair, nails</td>
<td></td>
<td>30</td>
<td>0.25</td>
<td></td>
</tr>
<tr>
<td>Saliva</td>
<td></td>
<td>30</td>
<td>0.25</td>
<td></td>
</tr>
<tr>
<td>Breath</td>
<td>Ammonia</td>
<td>50</td>
<td>0.4</td>
<td></td>
</tr>
<tr>
<td>Miscellaneous</td>
<td>Nasal mucous, semen</td>
<td>&lt;30</td>
<td>&lt;0.25</td>
<td></td>
</tr>
</tbody>
</table>

1 Data from Calloway & Margen 1971.

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**TABLE 2**

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Obligatory oxidative losses1</th>
<th>Ileal losses2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg/kg/d</td>
<td></td>
</tr>
<tr>
<td>Isoleucine</td>
<td>16.2</td>
<td>1.7</td>
</tr>
<tr>
<td>Leucine</td>
<td>27.4</td>
<td>3.2</td>
</tr>
<tr>
<td>Lysine</td>
<td>30.1</td>
<td>3.9</td>
</tr>
<tr>
<td>Threonine</td>
<td>15.5</td>
<td>4.2</td>
</tr>
<tr>
<td>Valine</td>
<td>16.9</td>
<td>2.9</td>
</tr>
<tr>
<td>Sulfur amino acids</td>
<td>13.6</td>
<td>1.8</td>
</tr>
<tr>
<td>Total</td>
<td>162</td>
<td>17.7</td>
</tr>
</tbody>
</table>

1 From Young et al. 1989.
2 From Fuller et al. 1994.

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**FIGURE 1** Metabolic pathways determining nitrogen balance. Nitrogen balance is the result of N intake (from the diet) and N losses, which consists of N recovered in urine and feces and miscellaneous losses.

**FIGURE 2** Nitrogen balance and nitrogen levels at four levels of nitrogen intake in healthy adult subjects (data from Price et al. 1994). A increase in the protein intake produces an increase in nitrogen losses via higher amino acid oxidation, especially in the fed state, and a trend toward positivation of the nitrogen balance.
balance when nitrogen intakes are increased (Price et al. 1994). Changes in the protein balance mainly result from the enhanced inhibition of protein breakdown by feeding and, to a lesser extent, from an increase in protein synthesis (Forslund et al. 1999) (Fig. 3). Whole body protein synthesis is probably sensitive to amino acid availability, whereas degradation may be sensitive to an interactive effect by both the amino acid level and insulin. The amplitude of diurnal body protein cycling increases, with no clear change in the mean daily protein turnover rate (Fig. 4) (Pacy et al. 1994). In addition, high protein intakes have been shown to result in a continuous, positive N balance of 1–3 g N/d, as shown in Table 3 (Cheng et al. 1978, Fisher et al. 1967, Forslund et al. 1999, Oddoye and Margen 1979, Price et al. 1994). However, it is not clear whether this apparent retention is real or originates from intrinsic errors in calculation of the N balance.

It is extremely important to determine whether the lean body mass can be increased by increasing the protein intake. Some recent results have strongly suggested that there probably is limited anabolic stimulation by high protein diets in healthy young adults. In contrast, this issue is particularly relevant in populations such as elderly persons, athletes or patients with protein-energy malnutrition. We studied the possibility of inducing an anabolic response by increasing the protein intake in moderately malnourished, elderly patients.

Our results showed that a shift from a spontaneous protein intake of 0.9 g protein/kg/d to 1.4 g protein/kg/d over a 10-d period using dietary supplements was associated in this population with an increase in both the fat-free mass and net protein synthesis, as measured using the 15N-glycine end-products method in both the postabsorptive and fed state (Table 3) (Bo, et al. 2000). The possible site of deposition under both acute and chronic feeding conditions remains to be determined. It is also unclear whether increasing the dietary protein level causes a generalized increase in protein turnover. Adaptability could be enhanced if the turnover was more rapid.

**Dietary protein nitrogen distribution during postprandial phase**

Dietary protein nitrogen and amino acids pass transiently through the nitrogen metabolic pools of the body. It is important to consider the diurnal cycle of feeding and fasting periods that result in postprandial dietary nitrogen gains and postabsorptive losses of body proteins (Millward et al. 1974). Acute nitrogen deposition during the postprandial phase is likely to be particularly critical in terms of the deposition of dietary protein in the tissues. Assessment of the postprandial utilization of dietary proteins is an appropriate approach, because this parameter is known to influence protein turnover (Marchini et al. 1993).

Studies have been undertaken to assess the acute postprandial utilization of dietary protein during the repletion phase of the diurnal cycle. The key steps concerning the fate of dietary nitrogen are considered to be i) the amount of nitrogen actually absorbed; ii) the amount that has been deaminated and recovered, mainly in the form of urea; and iii) the level of nitrogen retained in the body. The problems of measuring the postprandial utilization of dietary protein nitrogen in terms of ileal nitrogen digestibility and short-term retention of dietary protein nitrogen can be circumvented by the use of 15N-labeled proteins. This technique makes it possible to follow the metabolic fate of dietary nitrogen after its ingestion in humans (Bos et al. 1999, Gaudichon et al. 1999, Gausséres et al. 1996, 1997, Mahé et al. 1992, 1994, Mariotti et al. 1999).

Taking into account the different results obtained regarding evaluations of total nitrogen and protein metabolism in adult humans consuming 100–110 g/d of a well-balanced protein diet, of the 300 g/d protein turnover, we measured that 75–80 g was lost through the oxidative pathways and 14 g was lost at the ileal level (Fig. 5). The contribution of dietary nitrogen to the principal pathways was evaluated at 70–80 and 13–20 g/d, i.e., 30–40% and 17–25%, to anabolism and oxidative loss fluxes, respectively. This strongly suggests a preferential orien-
tation of dietary nitrogen toward anabolic pathways. This preferential orientation toward body protein synthesis is due to the adequacy of the dietary protein amino acid profile regarding the body protein and the compartmentation of protein metabolism. Indispensable amino acids supplied by dietary proteins equilibrate the free amino acid pool: the first-pass metabolism of dietary amino acids is mainly related to the splanchnic metabolism, whereas amino acids released from the peripheral metabolism are used in the catabolic pathways (i.e., alanine, glutamine).

To improve our understanding of the acute phenomena that occur after dietary nitrogen ingestion and due to limited access with the compartments of interest in human experiments, a compartmental modeling approach can also be used. Compartmental modeling enables simulation of the distribution of exogenous nitrogen in the major body nitrogen pools (including those not experimentally monitored) based on experimental measurements. This tool also allows a prediction of the future evolution of the system (Fouillet et al. 2000). This has been made possible by the development and validation of an 11-compartment model that makes a particular distinction between free and protein-bound amino acids in both the splanchnic and peripheral areas, to describe the cascade of transient metabolic processes that control the distribution of exogenous nitrogen throughout the body. The results obtained by modeling the pattern of dietary nitrogen distribution into the different body compartments after the ingestion of a protein meal is an useful tool to enable further definition of the notion of protein quality in a period of protein gain because it simulates the relative ability of a protein source to promote dietary nitrogen retention in different organs. It can also be used to discriminate between different nutritional conditions (type of the protein ingested, energy content of the meal) and to describe the processes involved in the differential metabolic utilization of various protein meals.

Nitrogen metabolism and dietary protein characteristics

The classic approach to measurement of net nitrogen retention is usually based on nitrogen balance data measured in subjects after adaptation to different protein levels over periods of several days (Millward and Pacy 1995, Munro 1964). Our current knowledge is somewhat limited. We know that diets containing poor quality protein are associated with an increase in nitrogen losses due to the inefficient utilization of indispensable amino acids. It is necessary to clarify which proportions of dietary and intestinal nitrogen are absorbed as amino acids or excreted in the feces, urine or other pathways and, most important, to determine which portion is utilized for protein synthesis and retained in body proteins.

One of the major limitations to the use of the classic nitrogen balance method is the existence of diurnal cycling for a transition between the fasted and fed states, which leads alternately to nitrogen postprandial accretion and postabsorp-

<table>
<thead>
<tr>
<th>Intake, g protein/kg/d</th>
<th>0.9</th>
<th>1.4</th>
<th>0.9</th>
<th>1.4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Turnover, g protein/kg/11 h</td>
<td>1.6 ± 0.3</td>
<td>2.1 ± 0.3*</td>
<td>2.2 ± 0.2</td>
<td>2.7 ± 0.2*</td>
</tr>
<tr>
<td>Synthesis, g protein/kg/11 h</td>
<td>1.4 ± 0.2</td>
<td>1.8 ± 0.2*</td>
<td>1.6 ± 0.2</td>
<td>2.1 ± 0.2*</td>
</tr>
<tr>
<td>Breakdown, g protein/kg/11 h</td>
<td>1.6 ± 0.3</td>
<td>2.1 ± 0.3*</td>
<td>1.2 ± 0.2</td>
<td>1.3 ± 0.2</td>
</tr>
<tr>
<td>Nitrogen excretion, mg N/kg/11 h</td>
<td>30.7 ± 9.6</td>
<td>50.3 ± 9.5*</td>
<td>88.6 ± 15.8</td>
<td>101.0 ± 14.4</td>
</tr>
</tbody>
</table>

1 Data from Bos et al. 2000. Values are means ± SEM.
2 *P < 0.05.
the ileal digestibility and metabolic fate of the individual protein source and the habitual level of protein intake influence the efficiency of postprandial dietary nitrogen accretion. As expected, the principal differences arise from modulations to the splanchnic fate of nitrogen. A protein source–dependent difference in interorgan amino acid metabolism has also been described in pigs after the infusion of either soy or casein (Deutz et al. 1998).

Protein and nitrogen homeostasis is achieved via a complex series of changes to the rates of whole body protein turnover, amino acid oxidation, urea production and nitrogen excretion that occur during the postprandial and postabsorptive periods of the day. The ability of humans to adapt to diets containing a wide range of protein levels has been the subject of considerable study, and guidelines have been drawn up regarding the minimum levels required to maintain health in the general population (FAO/WHO, 1985). Although it is clear that adult humans in different cultures survive on a broad range of protein intakes (from 0.6 g/kg/d to ≥ 3 times that amount), the effects of high protein diets are still poorly understood. Thus, the significance of amino acid oxidation still requires clarification in terms of its roles as nutrients used to provide energy. The ability of high protein diets to increase nitrogen retention and the protein turnover rate remains unclear. As far as an assessment of protein quality is concerned, it seems important to consider the level of protein in the diet. From a qualitative point of view, the standard methods used to evaluate protein mainly enable a discrimination between poor (unbalanced) and high quality protein diets. A comparison of relatively well-balanced protein diets remains difficult. However, in vivo methods are available that enable the accurate measurement of smaller differences between protein sources and the influence of other factors (other nutrients in the meal, habitual protein intake) on nitrogen metabolic utilization. The postprandial retention of dietary N could therefore represent a reference method for further validation of the PDCAAS method.

LITERATURE CITED


Using this approach, we calculated NPPU values of 80 and 72% for milk protein and soy protein, respectively, measured during the 8 h after the ingestion of a standard meal by healthy human subjects (Fig. 6). These data strongly suggest the existence of certain differences between the nutritional value of proteins. These differences should be taken into account when calculating amino acid scores. According to the method used at present, PDCAAS values that are >1 are rounded off to 1, based on the argument that (digestible) essential amino acid concentrations in a protein that exceed those in the reference amino acid pattern do not provide any additional nutritional value. The reality is probably more complex, and the best approach would probably be to compare different calculations of PDCAAS values with available in vivo results obtained in humans. Studies are in progress to determine both the ileal digestibility and metabolic fate of the individual 15N-amino acid ingested from milk, soy and wheat protein and to measure the NPPU of these proteins in subjects adapted to normal (1 g/kg/d) or high (2 g/kg/d) protein levels in their diet. As shown in Figure 7, preliminary results indicate that both the protein source and the habitual level of protein level

![Figure 7](image-url)

**Total nitrogen**

**Exogenous nitrogen**

**FIGURE 7** Total (left) and exogenous (right) levels of nitrogen excreted in urine 8 h after the ingestion of 30 g 15N-labeled milk, soy or wheat protein in humans after 7-d adaptation to normal (NP, 1 g protein/kg/d) or high (HP, 2 g protein/kg/d) protein diet (unpublished results).


