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An adaptive metabolic demand model for protein and amino acid requirements

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The shortcomings of the metabolic implications of the current protein requirements model are reviewed, and an alternative model, validated with [1-^{13}C]leucine balance results in human adults, is presented and evaluated in the context of defining protein requirements. The model identifies metabolic demands for amino acids as comprising a small fixed component and a variable adaptive component that is relatively insensitive to acute food or protein intake, but which changes slowly with a sustained change in intake, enabling N equilibrium to be achieved. The model accounts for the apparent low efficiency of utilisation of animal proteins in N balance studies and enables more realistic efficiency values to be measured within an experimental framework that takes account of the adaptive metabolic demand. However, the complex relationship between the adaptive metabolic demand and habitual level and quality of protein intake prevents prediction of protein quality by amino acid scoring, which can markedly underestimate actual values. In contrast to the current model, for fully adapted individuals risk of deficiency (i.e. negative N balance after complete adaptation) will only start to increase when intakes fall below the range of the true minimum requirements, i.e. a value that is currently unknown, but likely to be between 0·40 and 0·50 g/kg per d at the lower end of the reported distribution of requirements. At intakes greater than this with additional metabolic demands varying directly with intake, deficiency is only likely as a short-term response to a change to a lower intake within the adaptive range. Thus, for adults satisfying energy needs on most mixed human diets, intakes will be within the adaptive range, and N equilibrium ceases to be a useful indicator of nutritional adequacy of protein. In the context of prescriptive dietary guidelines it may be expedient to retain current values until the benefits (and any risks) of protein intakes within the adaptive range can be quantified. However, from a diagnostic perspective, indicators other than N balance need to be identified, since maintenance of N balance can no longer be used as a surrogate of adequate protein-related health.

Protein: Amino acids: Modelling

Defining human protein requirements has historically been difficult and consequently controversial. The general principle of the estimation of protein requirements has been to identify a maintenance requirement as an intake that would allow N equilibrium, plus additions for growth, pregnancy and lactation. Central to the design and interpretation of N balance and most other approaches to the evaluation of protein or amino acid requirements is adaptation. However, differences exist in the interpretation of balance data and especially on how considerations of adaptation should determine the analysis of N balance data and the construction of requirement models. The recently published US dietary reference intakes (Institute of Medicine, 2002) and the current FAO/WHO/United Nations University exercise to revise the 1985 report (Food and Agriculture Organization/World Health Organization/United Nations University, 1985) depend heavily on a new meta-analysis of all published N balance studies involving healthy adults (Rand *et al.* 2003). There was little discussion of adaptation in the dietary reference intakes report or in the recent meta-analysis of N balance data (Rand *et al.* 2003), consistent with the recent statements by one of the authors of the meta-analysis minimising the significance of adaptation in relation to the setting and application of nutrient requirements in human populations (Young & Borgonha, 1998). This is in marked contrast to arguments made by others. Sukhatme & Margen (1978) argued that an individual's protein requirement should allow for intra-individual variability of the requirement, i.e. adaptation, and therefore involve

Abbreviations: EAR, estimated average requirement; MD, metabolic demand; NPU, net protein utilisation; ONL, obligatory nitrogen loss; PPU, postprandial protein utilisation; RNI, recommended nutrient intake.

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definition of a range of intakes within which protein homeostasis could be maintained. More recently, Hegsted (2000) wrote: 'If the requirement of any nutrient is to be defined, the subjects must be allowed the time to adapt. Otherwise one simply estimates the nutrient supply in the current diet, which has little nutritional significance'. We have been arguing for some years now, initially on theoretical grounds (Millward & Rivers, 1988) and subsequently with some experimental support (Millward, 1998), that N balance and protein requirements can only be adequately explained from the perspective of a metabolic model within which adaptive components of the amino acid economy of the body are identified and accounted for. We now have sufficient experimental evidence to demonstrate that our model is essentially correct, and we present here the implications of an adaptive model of amino acid homeostasis in the context of defining protein requirements and identifying protein deficiency.

The current model of protein requirements

The current practice defines the protein requirement for maintenance as the intake that achieves N equilibrium as measured in multi-level N balance studies. This measure, the estimated average requirement (EAR), together with its inter-individual variation, can be used in a variety of prescriptive and diagnostic ways; it is most commonly expressed as a reference value (mean value+2SD), which represents the upper range (97·5th centile) of the distribution of the requirements, recommended daily allowance (Institute of Medicine, 2002) or recommended nutrient intake (RNI) (Department of Health 1991). The latter value can then be used either prescriptively or diagnostically as an intake that if consumed by an individual within a specified population group will result in very low risk of consuming less than the requirement.

Inherent to such considerations is that the between-subject variability in individual protein requirements is independent of any variability in protein intake. Thus, individuals consuming an intake equal to the average requirement have an equal probability of having a requirement that is higher or lower than the intake and therefore have a 50 % risk of protein deficiency (i.e. an intake less than the requirement). This is in contrast to current practice in relation to energy. In this case, recommended intakes are based on average values of the requirement, because the assumed correlation between metabolic demand (MD) and intake means that for those consuming average energy intakes, risk of deficiency is low and will only become important at intakes in the lower part of the intake distribution. Given the great importance of the assumptions made about how the protein requirement varies with dietary intake, for definition of dietary reference values and analysis of risk of dietary inadequacy it is important to examine the biological basis of N equilibrium and the nature of its intra-individual variation.

The biological basis of nitrogen equilibrium

A biological model for N equilibrium is implied by the linear statistical analytical model used to interpret N balance (Rand *et al.* 2003). The relationship between protein intake and N balance is described in terms of an intercept, representing the obligatory N loss (ONL) observed on a protein-free diet (a measure of obligatory MD) (Millward, 1998) and a slope, indicating the efficiency of dietary protein utilisation. Although it is generally recognised that N balance curves are non-linear, linear regression usually represents the simplest and most widely used analytical model, especially where some measure of between individual variability is required. The slope and intercept can be used to predict the intake for N equilibrium, i.e. zero balance, which is identified as the requirement:

$$\text{requirement} = \text{MD (intercept)}/\text{efficiency of utilisation (slope)}.$$

The meta-analysis of Rand *et al.* (2003) brings together most N balance studies on adults. From these they identified a subset of nineteen studies reporting results involving at least three test protein intake levels in each of 235 individual subjects. These were analysed by linear regression for each individual after adjustment for either 4·8 or 11·0 mg N as surface losses (in temperate or tropical climates) for those studies reporting only urine and faecal N losses. Median values for slope, intercept and the requirement are reported. Median values of all individual studies (*n* 235) were 0·47 and 48·00 mg N/kg per d for slope and intercept defining a requirement of 105 mg N (0·65 g protein)/kg per d. The biological implication of this is that human adults have a protein requirement for maintenance of about twice that of the obligatory MD because of their inefficiency (only 50 %) of protein utilisation.

The extent and nature of the variability in measured values of the protein requirement

Rand *et al.* (2003) employed an operational approach to determine a CV of 12 % for the inter-individual variability from assumptions about the extent and nature of the various components of variability after trimming 5 % of the data. This defined a RNI for protein as 130 mg N (0·83 g protein)/kg per d. Without any attempt to partition the variability, the CV would be much higher, with a RNI of about 1·2 g protein/kg per d. Given the somewhat arbitrary assumptions made about partition of variability the question can be posed as to whether a significant part of the variability could be explained by variability in the extent to which individuals or groups could have fully adapted, i.e. adjusted their rates of amino acid oxidation, urea production and protein synthesis and proteolysis to the low-protein test diets fed in the balance studies.

The difficulty in addressing such a question lies in separating such influences from the range of known confounding influences on both measurement and achievement of N balance. One such confounder is the non-linearity of the balance curve (see Millward & Roberts, 1996), with studies conducted at low intakes likely to have steeper slopes and more negative intercepts and to underestimate requirements, compared with studies conducted with supra-maintenance intakes, which will tend to have

shallower slopes, less negative intercepts and to overestimate requirements. In the reported data set, there is an equal contribution of variability in the slopes and intercepts and the two values are inversely correlated (r −0·86). Since there is no obvious biological reason why efficiency of utilisation and obligatory MD should be inversely correlated, an analytical explanation associated with linear regression of non-linear data is more likely. A second confounder is the marked sensitivity of N balance to energy balance. According to the multiple regression of N balance on energy intake and N intake reported by Pellet & Young (1992), two-thirds of the variability reported in the meta-analysis of Rand *et al.* (2003) (SD 31·9 mg N/kg) could be accounted for by an error of only about ±0·2 of BMR in estimating the true energy needs of a subject.

With measurement and design problems as severe as this, it is difficult to evaluate the likely influence of incomplete adaptation. The possibility of incomplete adaptation is a feature of the design of most N balance studies with diets randomised on the assumption that the order in which low-protein test diets are fed can influence the outcome. When diets are not randomised, different N balance curves are obtained according to the order in which intakes below habitual are fed (Atinmo *et al.* 1988) implying that 2 weeks is not long enough. However, within the database analysed by Rand *et al.* (2003), there were no discernable differences in outcomes according to the likely variation in habitual diets of the subjects (e.g. studies in developing as opposed to developed countries). Nevertheless, by the same token, background diets were in fact unknown, so that it cannot be excluded that the short-term balances of these studies were not long enough to allow full adaptation to the test diets and that variation in the habitual protein intakes of subjects could account for much of the variability within and between studies. Were this true, it affords a mechanism whereby protein intakes and requirements may be correlated, profoundly altering the way in which risk of deficiency is calculated from requirement and intake values.

Is the current requirements model biologically sensible?

Within the classical model of protein utilisation, the characteristic of a particular protein source that determines the efficiency of its utilisation is net protein utilisation (NPU), defined in terms of digestibility and biological value: the latter reflects utilisation of absorbed protein as influenced by the pattern of the absorbed amino acids relative to that of the MD. This model was consistent with practical findings for protein utilisation in the rapidly growing laboratory animal, where animal proteins such as egg or milk had NPU values that approached 1 (e.g. Platt *et al.* 1961). In contrast, human studies on adults indicated from the outset that such proteins exhibited NPU values significantly <1. The results reviewed by Rand *et al.* (2003) indicate apparent NPU values (slopes) of 0·46 for high quality animal protein and values of 0·47 and 0·48 for vegetable and mixed protein sources. This implies that in healthy adults protein utilisation is low with no influence of dietary source on utilisation. Given that when different protein sources are compared within the same study, differences when observed are very small (Millward *et al.* 1989), it is not surprising that in an aggregated analysis no significant difference can be identified. However, the important question is why the mean value for the slope should be so low.

Millward & Rivers (1988) predicted, mainly on theoretical grounds, that amino acid requirements are best described by a model including adaptive changes in amino acid oxidation that influence N losses and result in an apparent inefficiency of utilisation. Since that time, however, we have reported a series of [^{13}C]leucine balance studies that confirm an adaptive MD model and provide a framework for the interpretation of N balance data.

An adaptive metabolic demands model of protein requirements

The metabolic focus of the model is the adaptive behaviour of amino acid oxidation as a determinant of the MD. Some experimental justification of the model has been summarised (Millward, 1998). Fig. 1 shows the model in terms of the fate of dietary protein (Fig. 1(a)) and in relation to balance regulation during the diurnal cycle (Fig. 1(b)).

The MD consists of two components, obligatory and adaptive. The obligatory MD includes non-essential N

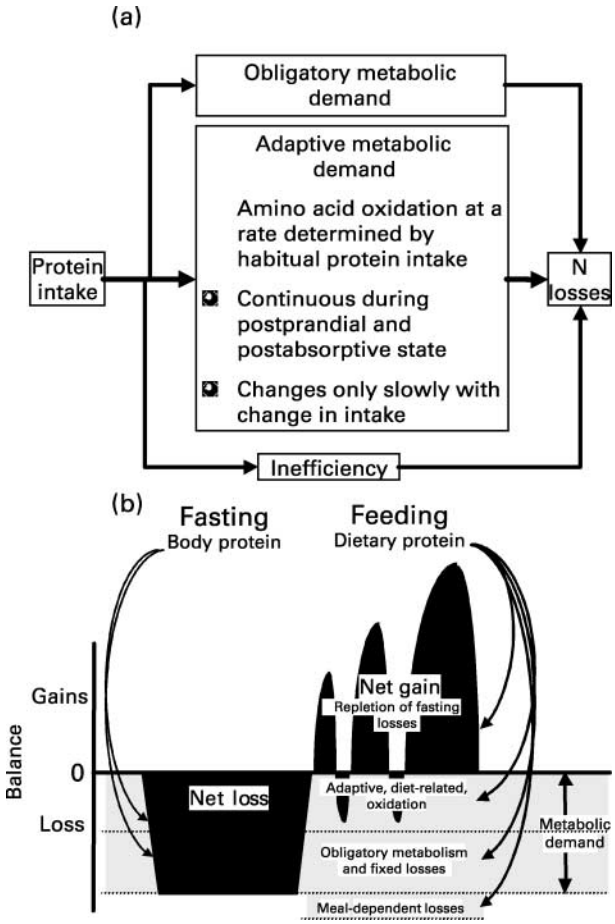

Fig. 1. Adaptive metabolic demands model of the protein requirements. The model is shown in terms of the fate of dietary protein (a) and in relation to balance regulation during the diurnal cycle (b).

and conditionally essential and indispensable amino acids that act as precursors for any net protein synthesis, for non-protein products derived from amino acids (especially phenylalanine, tryptophan and methionine) and any N lost in the large bowel, all of which give rise to nitrogenous excretory endproducts (see Reeds, 2000). The overall magnitude is indicated by the ONL. On the basis that the ONL (47 mg N/kg; see Rand *et al.* 2003) represents loss of tissue protein (0·293 g protein/kg) to provide sufficient of the rate-limiting indispensable amino acid (i.e. the amino acid with the highest ratio of amount as a proportion of the obligatory MD/amount as a proportion in tissue protein mobilised to provide the ONL), it can be predicted that for each amino acid, except the rate-limiting one, the obligatory MD will be less than that contained in 0·293 g tissue protein. However, results from studies on animals show the maintenance pattern to differ from that for growth, with lower levels of lysine and leucine, with the S amino acids or tryptophan being rate-limiting and with the major component being for non-essential N (Millward & Rivers, 1988; Millward, 1998). Certainly in human subjects, N losses on a methionine-free diet are much greater than on a leucine-free diet (Raguso *et al.* 1999).

The adaptive MD involves amino acid catabolism that occurs in the fed and postabsorptive state at a rate that varies mainly with the habitual protein intake. It is relatively insensitive to acute food or protein intake, changing only slowly with a sustained change in intake (e.g. Oddoye & Margen, 1979). One possible metabolic explanation of this is that during slow growth or at weight maintenance, in order to be able to rapidly dispose of dietary protein in excess of minimal needs and maintain the very low tissue concentrations of the potentially toxic branched chain, aromatic and S amino acids, the capacity of the pathways of oxidative catabolism of these particular amino acids adapts to match the habitual protein intakes (Millward & Rivers, 1988; Millward, 1998). Although these pathways are to some extent regulated by feeding and fasting, this regulation is incomplete, so that amino acid oxidation continues to occur after dietary protein is disposed of, continuing in the postabsorptive state with net catabolism of tissue protein. The consequences of this for N homeostasis is a diurnal cycle of fasting losses and fed-state gains of increasing amplitude with increasing habitual intake. This has been demonstrated with both 12 h N balances and short-term [1-^{13}C]leucine balances in subjects fed a wide range of protein intakes (Pacy *et al.* 1994; Price *et al.* 1994; Quevedo *et al.* 1994; Millward, 1998). The apparent protein (or amino acid) requirement is the intake required to balance losses for an individual at their level of adaptation to their habitual diet.

Validation of the adaptive metabolic demands model

On the basis of the model in Fig. 1(b), MD, efficiency of utilisation and apparent protein and amino acid requirements can be assessed during acute measurements of amino acid or N balances during the fasting–feeding transition (Millward & Pacy, 1995). Thus, postabsorptive losses (24 × rate per h) indicate the MD at the level of adaptation of the subject; the change in balance in response to feeding an intake similar to the habitual intake indicates the efficiency of postprandial protein utilisation (PPU) and the apparent protein requirement (i.e. the intake required to balance losses at the level of adaptation to the habitual diet of the subject) is indicated by MD/PPU. If the model is correct, then an individual's apparent requirement will vary with intake because their MD, indicated by postabsorptive losses, will vary with habitual intake. In addition, PPU should indicate values for different protein sources that are consistent with what might be expected, i.e. very high values for animal proteins and lower values for amino acid-limited sources such as wheat. In fact, the expected value for PPU is not entirely straightforward, since the overall amino acid composition of the MD for maintenance is uncertain. Thus, within the model (Fig. 1(b)), food protein provides for both net protein deposition, of which the amino acid composition will be that of mixed tissue protein, and the obligatory and adaptive oxidative losses, the amino acid composition of which is not known.

Results published by our group that are derived from both 12 h N balances and [1-^{13}C]leucine tracer balances are shown in Table 1. The tracer balance results are best interpreted by predicting N balance from leucine balance and calculating PPU as an alternative to NPU (as in Millward *et al.* 2000, 2002). The current database is small, but it is clear that MD and the apparent protein requirement do change in subjects after short-term (2 weeks) adaptation to varying protein intakes (although it cannot be assumed that these adaptive changes are complete), that milk and egg protein is utilised with a very high efficiency and that wheat protein utilisation is considerably less efficient than that of milk, although more efficient than predicted. In addition, in the elderly, whilst PPU is not different from that in younger adults, the MD and consequent apparent protein requirement is lower than in younger adults (see Fereday *et al.* 1997; Millward *et al.* 1997).

The main uncertainties with this approach are: (1) the [^{13}C]leucine balances involve measurements over short periods of a few hours and the results are then extrapolated to 12 or 24 h; (2) a conversion factor must be used to predict N balance from leucine balance and the magnitude of this needs careful consideration (Millward *et al.* 2002). Thus, there is some uncertainty in the values shown in Table 1. However, the extent of any error is indicated by comparisons of leucine oxidation with N excretion; this showed that although [^{13}C]leucine oxidation in this protocol tended to underestimate N balance, the two measures were proportional over a wide range of protein intakes (Price *et al.* 1994). Thus, any error in the magnitude of the MD will not invalidate the conclusion that the MD is variable with protein intake, and that PPU values, measured by either N balance or leucine oxidation in the same subjects, are quite similar.

One important additional caveat for the PPU representing a true measure of protein utilisation relates to experimental design. The fasting–feeding transition from negative to positive N balance involves responses to energy as well as protein, so that it is not a true measure

Table 1. Measurements of metabolic demands, efficiency of protein utilisation and the apparent protein requirement

	Intake		Metabolic demand (g protein/kg per d)†		PPU‡		Apparent requirement (g protein/kg per d)§	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Influences of protein intake*								
N balance								
	0·36	0·004	0·58	0·09[a]	0·95	0·14[a]	0·62	0·06[a]
	0·77	0·034	0·74	0·11[b]	0·81	0·10[a]	0·92	0·12[b]
	1·58	0·096	1·23	0·25[c]	0·81	0·12[a]	1·52	0·17[c]
	2·31	0·608	1·74	0·47[d]	0·87	0·11[a]	2·02	0·55[d]
[^{13}C] Leucine balance								
	0·40	0·03	0·43	0·08[a]	1·03	0·50[a]	0·53	0·34[a]
	0·77	0·04	0·57	0·10[b]	0·92	0·28[a]	0·65	0·15[b]
	1·51	0·08	0·85	0·12[c]	0·94	0·06[a]	0·91	0·16[c]
	2·00	0·13	1·06	0·22[c]	1·00	0·08[a]	1·08	0·29[c]
Age and gender effects‖								
Young adult (F)			0·83	0·14[a]	0·99	0·07[a]	0·79	0·15[a]
Young adult (M)			0·90	0·06[a]	1·05	0·10[a]	0·80	0·07[a]
Middle-aged (M)			0·87	0·18[a]	1·01	0·05[a]	0·79	0·18[a]
Elderly (F)			0·52	0·14[b]	0·92	0·16[a]	0·57	0·20[b]
Elderly (M)			0·58	0·16[c]	1·05	0·13[a]	0·53	0·15[b]
All			0·74	0·21	1·00	0·12[a]	0·70	0·20
Protein quality								
Multiple small meal protocol¶								
Milk			0·73	0·18	1·00	0·09[a]	0·75	0·18[a]
Wheat			0·73	0·18	0·68	0·06[b]	1·07	0·20[b]
Single large meal protocol**								
Milk			0·81	0·16	0·93	0·02[a]	0·87	0·17[a]
Wheat			0·81	0·16	0·61	0·03[b]	1·31	0·23[b]

PPU, postprandial protein utilisation; F, female; M, male.
[a,b,c]Mean values within a column with unlike superscript letters were significantly different ($P \leq 0·05$).
* Data for healthy adults recalculated from Price *et al.* (1994) involving either [^{13}C]leucine or N balances in the fasted and fed state. Diets fed during the balance periods contained protein of which 80–100 % was either dairy- or egg-based.
† Calculated from postabsorptive N or leucine losses scaled to 24 h assuming that leucine oxidation represents an equivalent loss of tissue protein nitrogen at 4·77 mg leucine/g N and that the total amino acid–N conversion factor is 7·31 (Millward *et al.* 2002).
‡ Fractional efficiency of protein utilisation (utilisation/intake) calculated from ΔN or leucine balance/ΔN or leucine intake.
§ Apparent dietary requirement for daily balance, calculated as the metabolic demand/PPU.
‖ Recalculated from Fereday *et al.* (1996) and Millward *et al.* (1996). Studies involved changes in N balance calculated from [1-^{13}C]leucine balance during the transition from a low to a high protein intake in subjects fed repeated small milk-based meals.
¶ Data from Millward *et al.* (2000) measured with the same protocol as in footnote *.
** Data from Millward *et al.* (2002) calculated from changes in N balance calculated from [1-^{13}C]leucine balance during the fasted–fed transition in subjects fed a single large meal of 50 g protein.

of protein utilisation *per se*. Indeed, feeding a protein-free meal reduces amino acid oxidation and N excretion, improves balance with zero intake and gives an infinitely large value for PPU. PPU more closely indicates the utilisation of protein when measured with a three-phase (fasting, low-protein, high-protein) protocol during the transition between isoenergetic low- and high-protein meals (Gibson *et al.* 1996; Fereday *et al.* 1997, 1998; Millward *et al.* 2000).

To date, few other investigators have adopted these methods to quantify protein utilisation from tracer balance studies. One exception is Tomé, who has been developing methods using ^{15}N to assess the transfer of ^{15}N from intrinsically ^{15}N-labelled proteins into urea (Tomé & Bos, 2000), calculating a modified PPU value, the net PPU (net PPU = ^{15}N ingested − (^{15}N ileal + ^{15}N body urea + ^{15}N urine)/^{15}N ingested). He reports values of 72 and 80 for soyabean and cows' milk. However, this approach will underestimate protein utilisation, since no distinction is made between N loss through consumption within the MD and loss as a true inefficiency of utilisation (see Fig. 1). In addition, the considerable postprandial amino acid-N exchange between the dietary and endogenous amino acids will reduce apparent differences in PPU between different dietary protein sources.

Time course of changes in adaptive metabolic demands

A feature of the response to changes in protein intake are gains and losses of body N (e.g. Oddoye & Margen, 1979), identified in the past as the labile protein reserves, but largely unexplained (see Garlick *et al.* 1999) apart from some changes in the body urea and free amino acid pools and possible changes in the size of those splanchnic organs, which vary with functional demand, such as the hepatic protein mass (Waterlow *et al.* 1978; Millward, 1995). The labile protein reserves can be identified in metabolic–kinetic terms, as the consequence of the insensitivity of the adaptive MD to altered intake, i.e. changes in cellular protein through delay in the regulatory changes in protein turnover, amino acid catabolism and N excretion, which need to occur to allow N equilibrium after a change in protein intake. Such equilibrium can clearly be achieved over a wide range of intakes eventually, as

shown by the stability of human adult body weights achieved on a wide range of protein intakes. However, it is clear that alterations of intake within the normal range can result in large losses in the short term (Oddoye & Margen, 1979). N balance and stable isotope studies in human subjects and animal studies of measurement of enzyme activities of amino acid catabolism and the urea cycle (see Waterlow, 1999) provide abundant evidence that this adaptation happens slowly, requiring at least several weeks in human subjects. In studies of the ONL, subjects fed a protein-free diet took between 10 and 17 d to achieve a constant low level of urea N excretion (Scrimshaw *et al.* 1972). In studies involving diets in which protein intakes were reduced from adequate to 0·35 g/kg per d, men took 7–28 d to achieve N equilibrium (Durkin *et al.* 1981). The subjects studied by Oddoye & Margen (1979) took 16–40 d to achieve balance after the reduction in intake from 3 to 1 g protein/kg per d, and presumably would have required an even longer period to replete the losses incurred during the transition.

We reported [^{13}C]leucine oxidation and 12 h N balances studies of the time course of the adaptation to a reduction from a habitual intake of 2·00 to 0·77 g/kg per d over 9–14 d, which showed the negative balance to reflect a lag in reduction of both postabsorptive and postprandial N losses (Quevedo *et al.* 1994). The changes with time in overall N balance and in the apparent protein requirement, calculated from postabsorptive N losses and from PPU (ΔN balance/ΔN intake), are shown in Fig. 2. After the reduction in intake, the apparent protein requirement fell only slowly from 1·70 g protein kg/d at the start of the study, a value quite similar to the intake (1·90 g/kg per d) to 1·20 g protein/kg per d at 9 d, considerable higher than the intake of 0·77 g/kg per d, consistent with slow adaptation. The incomplete adaptation involved both an insufficient fall in MD (postabsorptive losses) and a fall in PPU from 0·89 to 0·70 due to excessive postprandial activation of amino acid catabolism.

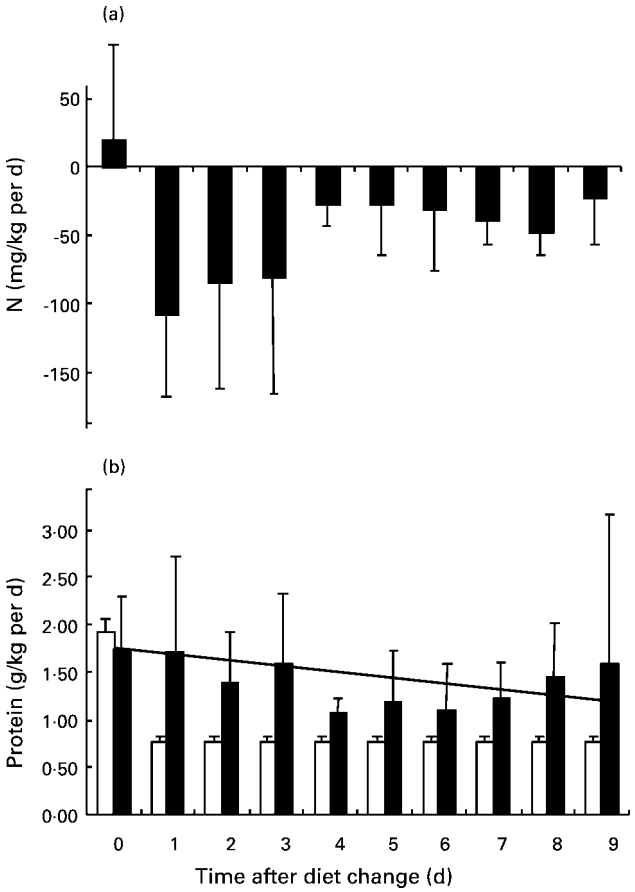

Fig. 2. Time course of the adaptive changes in nitrogen balance and the apparent protein requirement after a change in intake from 2·00 to 0·75 g/kg per d. Results shown as daily nitrogen balance (a) and changes in the apparent protein requirement calculated from postabsorptive nitrogen losses and from postprandial protein utilisation as indicated by change in nitrogen balance/nitrogen intake (b). □, Intake; ■, apparent requirement; —, linear regression of apparent requirement *v.* time. Results recalculated from Quevedo *et al.* (1994).

Minimum intakes for nitrogen equilibrium

The key issue is the lower limit of successful adaptation at which an appropriate body composition can be maintained, i.e. the minimum intake for N equilibrium. If the assumption is made that variable degrees of adaptation to the low-protein test diets fed in short-term balance studies accounts for a significant part of the variability observed within and between studies, then the values observed at the lower limits of the requirements distribution should give some indication. Within the thirty-two separate multilevel balance studies analysed by Rand *et al.* (2003), ten studies have median values $<0{\cdot}60$ g/kg per d, five studies have median values $<0{\cdot}50$ g/kg per d with the lowest at 0·42 g protein/kg per d, whilst of the total number of individual measurements after trimming for outliers (n 224; minimum 0·34, maximum 1·13 g protein/kg per d), thirty-six individuals (15 %) had requirements $\leq 0{\cdot}50$ g protein/kg per d. This means, as shown in Fig. 3, that the lower range of the requirement estimates approaches the upper range of the values for the ONL. However, whether requirement estimates as low as this represent intakes that would allow successful adaptation, allowing maintenance of body N equilibrium without cost in the long term has always been controversial and is unresolved in terms of experimental evidence with long-term balance studies.

Yoshimura *et al.* (1972) tested intakes of 0·77 g protein/kg from cereal and pulses, and 0·57 g protein/kg from meat and eggs in 12-week N balance study of protein requirements: although N balance tended to be positive there were decreases in BMR, haemoglobin and urinary 17-keto-steroids and 17-hydroxycorticosteroids, with increased serum antidiuretic hormone. They concluded that N balance alone could not serve as an adequate criterion of dietary adequacy. In contrast, Calloway & Chenowweth (1973) reported that 0·61 g milk protein fed for 3–5 weeks maintained N balance and body weight with no fall in haemoglobin. The most extensive series of long-term balances (Garza *et al.* 1977*a*,*b*, 1978) on intakes equal to the 1973 safe allowance (0·57 g egg protein; Food and Agriculture Organization/World Health Organization, 1973) showed negative N balance, loss of lean body mass and deteriorating values for serum protein and serum transferases unless additional

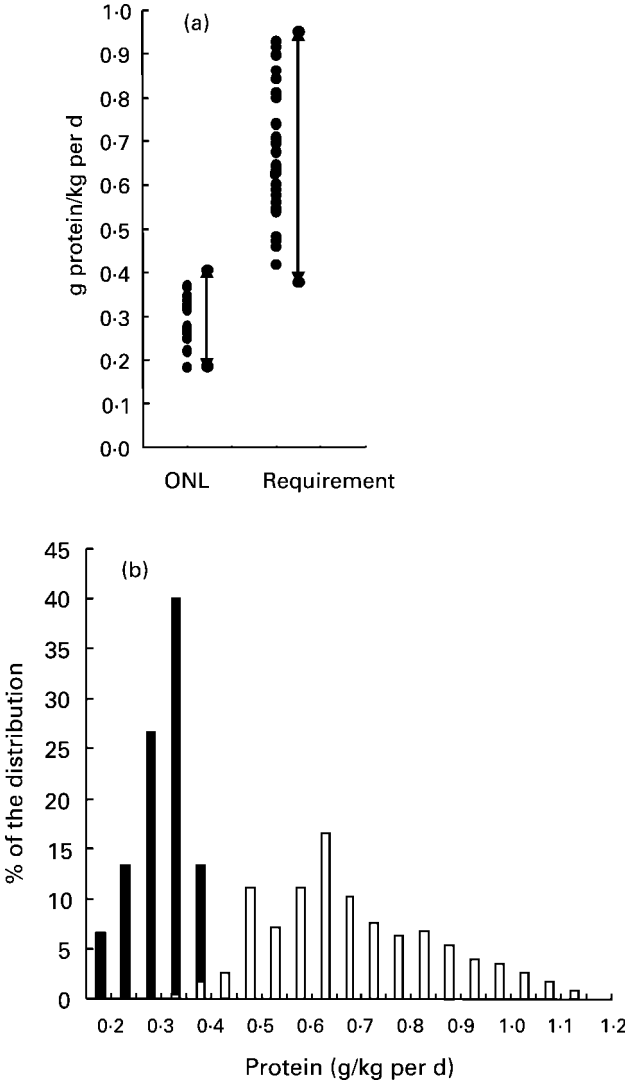

Fig. 3. Range and distribution of nitrogen balance studies of the protein requirement and obligatory nitrogen loss (ONL). Reported values for the ONL and the intakes for nitrogen equilibrium (expressed as protein equivalents) from the meta-analysis of nitrogen balance data reported by Rand *et al.* (2003). Values are: (a), medians and overall range for studies reported by Rand *et al.* (2003) (↔ , ± 2 SD (*n* 15 for ONL, *n* 32 for requirement)); (b), distribution of individual values (□, requirement; ■, ONL).

energy or non-essential N was fed. The only study to examine intakes in the lower range of the short-term balance shown in Fig. 3 was a study reported to the FAO/WHO/United Nations University meeting in 1981 (Durkin & Margen, 1981) involving 77 d N balances in six subjects fed a very low intake of egg protein (approaching the ONL: 57 mg N/kg, 0·36 g protein/kg) and confined to a metabolic unit. There were no adverse physiological (work performance) or biochemical changes, and after a variable period of obvious negative balance (7–28 d), all subjects achieved a stationary N balance state, which involved N equilibrium for four subjects and a small negative balance (7 or 10 mg N/kg per d) for two others. However, most of the subjects lost weight during the study and since no measurements were made of body composition, the extent of lean tissue loss was not indicated.

A further more complex issue was raised by these latter studies. This involves cyclical patterns in N excretion for five of the six subjects in the studies as identified by a statistical procedure that examined the extent of serial correlation. This was consistent with previous reports by Sukhatme & Margen (1978). This was interpreted as indicating a regulatory mechanism matching intake to losses and indicating an 'adequate' intake. In contrast, Rand *et al.* (1979) analysed all long-term balance studies at the Massachusetts Institute of Technology (Boston, MA, USA) for serial correlations and were unable to identify them in most of their subjects, at least after correcting for long-term trends, concluding that variations in urinary N output were random. However, the key differences between these two sets of long-term balances were the magnitude of the minimum intake at which N balance can be achieved at no obvious cost, i.e. 0·35 or > 0·60 g protein/kg per d. Clearly, even though N balance was eventually achieved without adverse biochemical changes, the weight loss at the lower intake leaves the issue of the minimum protein intakes for N equilibrium unresolved, as is the extent to which those short-term balance studies indicating requirements < 0·50 g protein/kg per d represent the lower limits of adaptation, and it is likely to remain so, at least in terms of experimental data.

Whilst it has often been argued that information already exists in terms of the nutritional status of populations consuming very-low-protein diets habitually, such results are unlikely to resolve the issue. This is because intakes as low as this in subjects consuming their energy needs are rare and only possible on relatively unsupplemented starchy root-crop staples (see Millward, 1999*a*). As shown in Table 2, for a 70 kg young adult male consuming a variety of either cereal or starchy roots, tubers or fruit staples as sole sources of energy at the rate of 1·75 × BMR (183 kJ (43·7 kcal)/kg), while cereals and potatoes provide digestible protein intakes between 0·76 and 1·80 g/kg per d, only a few non-cereal staples provide intakes substantially less than 0·50 g/kg per d (i.e. Ethiopian banana (Ensete) 0·36, plantain 0·35, cassava 0·23 g/kg per d). Furthermore, such diets will almost certainly be nutritionally limiting in several key micronutrients that would impact on most indicators of nutritional status.

Implications of adaptive metabolic demands model of protein requirements

The apparent inefficiency of protein utilisation in multi-level nitrogen balance studies is explained

The mean adult requirement value of 0·66 g/kg indicated by the N balance studies implies an overall efficiency of utilisation of dietary proteins of about 50 % in replacing the ONL. This value results from linear regression of data that is probably curvilinear, so that in practice it can be assumed that the derived efficiency of 50 % is the mean of values that fall from a high value at low intakes to a low value that approaches zero at the asymptote. Fig. 4 shows a schematic representation of the assumptions behind an NPU of protein utilisation in studies at which varying intakes are fed and N balance measured.

Table 2. Protein intakes from staples consumed on their own to provide energy intake equivalent to BMR × 1·75 in 70 kg male subjects*†

	Protein intake (g/kg per d)	
	Total	Digestible‡
Cereals		
Oats	1·98	1·78
Hard wheat	1·95	1·76
Soft wheat	1·84	1·66
Ethiopian teff	1·83	1·65
Ukraine Armenian Republic wheat	1·50	1·35
Rye (wholegrain)	1·37	1·23
Sorghum (average of all varieties)	1·36	1·22
Millet (dried)	1·33	1·20
Maize (yellow)	1·20	1.08
Rice (milled and polished)	0·84	0·76
Starchy roots, tubers and fruits		
Potatoes	0·91	0·82
Taro	0·77	0·69
Hausa –potato (tuber)	0·60	0·54
Breadfruit pulp	0·60	0·54
Yam	0·59	0·53
Sweet potato	0·58	0·52
Ethiopian banana (ensete) flour	0·40	0·36
Plantain (ripe)	0·39	0·35
Cassava (dried)	0·26	0·23

* Source of data was food composition tables for use in Africa (Food and Agriculture Organization, 1968). Values for moderately active (physical activity level 1·75), 56 kg women would be 7 % lower.
† 183 kJ (43·7 kcal)/kg.
‡ Assuming digestibility of 90 % in each case.

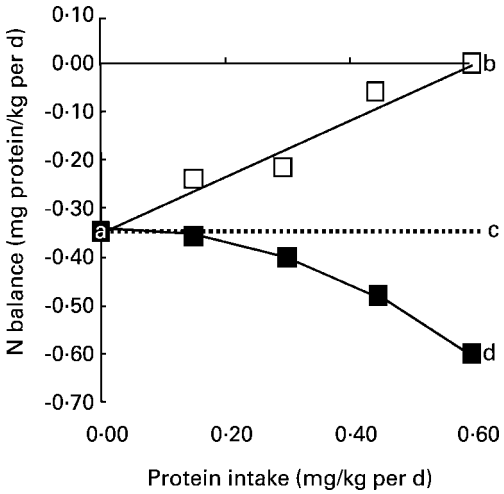

Fig. 4. Actual and assumed values for the metabolic demand (MD) in multi-level nitrogen balance assays of net protein utilisation (NPU). □, Assumed MD; ■, actual MD; —, balance. The slope assay of NPU equates the slope of the nitrogen balance curve (b−c/a−c) with NPU. This assumes that the MD is constant and equal to the zero intake intercept (the obligatory nitrogen loss). However, with an adaptive MD model, the actual MD and instantaneous postabsorptive losses will increase with intake. Thus, the nitrogen balance curve will underestimate the efficiency of utilisation and the true efficiency will be indicated by b−d/a−c. The magnitude of the MD and the consequent shape of the balance curve has been arbitrarily drawn assuming an adaptive MD that would result in an apparent overall MD equivalent to 0·60 g protein.

The slope of the balance curve indicates net protein utilisation, but only if the maintenance requirement is constant (i.e. =ONL) at each level of intake as usually assumed.

In the rapidly growing animal, disposal of dietary amino acids can occur through growth, i.e. net protein synthesis, so that an adaptive oxidative catabolism component of the requirement will be small or undetectable and the slope of the balance curve, usually in the positive balance range, will be similar to the true NPU. However, in studies of human subjects there is a significant adaptive component of the MD. Thus, the losses that would occur acutely if no protein were fed, which is a measure of the total MD, increase with intake. Thus, the true efficiency will be higher, since the Δbalance term, the difference between observed balance and true MD (Fig. 4(b, c and d)), will be greater than that calculated assuming a constant MD (Fig. 4(b and c)) Confirmation of this is indicated by the high efficiency of utilisation of high-quality protein shown in Table 1.

The assessment of protein quality by amino acid scoring is problematic

Because the relationship between the MD for indispensable amino acids and N and the habitual level and quality of protein intake is complex, identification of a reference scoring pattern also becomes complex. In this model, with increasing dietary protein intake increasing net protein deposition occurs with feeding to replace the increasing postabsorptive losses. It might be assumed that the need for higher protein quality increases with protein intakes, because the adaptive MD will increasingly involve an amino acid pattern like that of tissue protein. Indeed, Young & El-Khoury (1995) have used this argument to justify the validity of the Massachusetts Institute of Technology requirements pattern. In his considerations of these issues, Waterlow (1996) concluded: 'It follows that conceptually the indispensable amino acid requirement pattern will be some kind of halfway house between the pattern of irreversible losses and the pattern of body protein, and the relative proportions of the two patterns will be influenced by the level of protein intake.' In fact, the experimental evidence suggests a complex relationship between essential amino acid requirements and the habitual level and quality of protein intake with adaptive mechanisms that compensate for low protein quality (see Millward, 1998).

First, the amount of postabsorptive tissue protein loss to be replaced by the diet will depend on the extent of the true postabsorptive state during a day: i.e. individuals with a multi-meal eating pattern from an early breakfast to a late supper may spend less time in a true postabsorptive state, mobilising less tissue protein than single large-meal eaters. For a patient in overall balance who is fed continuously, there will be no requirement for net replacement of tissue protein so that the requirement pattern should closely reflect the non-protein MD.

Second, some amino acids liberated during the postabsorptive net proteolysis and negative N balance may not be completely oxidised (e.g. lysine and threonine), allowing recycling for net protein synthesis during PPU.

This is suggested by the observation of a higher than expected efficiency of wheat protein utilisation measured during [1-^{13}C]leucine balances (Millward *et al.* 2000, 2002).

Third, the amplitude of diurnal cycling and the need for postprandial protein deposition may be adaptive according to the amino acid composition of the diet. This is suggested by increasing postabsorptive losses ([1-^{13}C]leucine balances) in subjects fed isonitrogenous diets with increasing indispensable amino acids (Marchini *et al.* 1993) or with increasing leucine content (El Khoury *et al.* 1994*a*,*b*). Kurpad & Vaz (2000) pointed out the variability in 12 h fasted leucine oxidation with the level of the prior 6 d leucine intake. Thus, these results point to substantial adaptive reductions in the amplitude of diurnal cycling and in the consequent MD for indispensable amino acid in response to reductions in dietary protein quality.

Together, these results suggest a complex adaptive response to varying intakes of N and amino acids. This is in contrast to the traditional model in which there is a fixed and measurable amino acid requirement pattern, which allows biological value to be determined by amino acid scoring and utilisation predicted from the protein digestibility-corrected amino acid score method (Food and Agriculture Organization/World Health Organization, 1991). From the perspective of the adaptive MD model, the utilisation of protein that might be achieved as part of a habitual diet can only be predicted in a very approximate way. This means that direct study by balances with body weight and composition measurements are required in order to establish the utilisation with any certainty.

Identification of dietary reference values for protein and assessment of risk of protein deficiency requires reconsideration

With protein intakes and requirements assumed not to be correlated, the risk of deficiency for an individual will rise as protein intake falls below the 97·5th centile of the requirements distribution (i.e. mean value + 2 SD), reaching 50 % at an intake equivalent to the average requirement value. In fact, as is apparent in Fig. 3, the distribution of individual requirements for protein, as reviewed by Rand *et al.* (2003), is not normal but is skewed, so that its calculated SD and CV do not have the usual intuitive meaning. In the application of these results in the dietary reference intakes report (Institute of Medicine, 2002), the EAR is calculated as the median value and an approximate SD is calculated (equal to a CV 12 %), giving a recommended daily allowance of 1·24 × EAR. On this basis, calculation of risk of deficiency will be as shown in Fig. 5(a). However, with an MD comprising a quite small fixed component and an adaptive component that varies with intake, then calculation of risk will be quite different. For fully adapted individuals, risk of deficiency (i.e. negative N balance after complete adaptation) will only start to increase when intakes fall below the upper range of the true minimum requirements, i.e. a value that is currently unknown but which is an intake considerably below the current EAR and at the lower end of the reported distribution of requirements, possibly approaching that

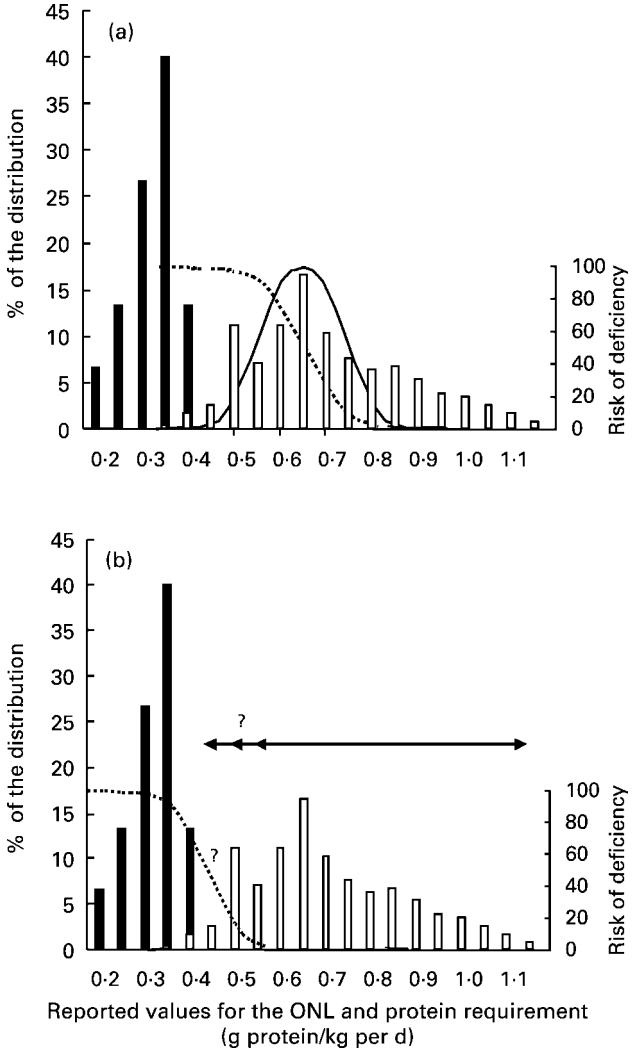

Fig. 5. Distribution of requirements and calculation of risk of deficiency for an individual for current (a) and adaptive metabolic demands model (b) of protein requirements. ↔, Adaptive range. Values are the reported values for the obligatory nitrogen loss (■; ONL) and intakes for nitrogen equilibrium (□; expressed as protein equivalents) from the meta-analysis of nitrogen balance data reported by Rand *et al.* (2003). (a), On the basis of the median value (0·65 g/kg per d) and estimated between individual variation (CV 12 %), a normal distribution of requirements is shown with risk of deficiency for an individual (- - -) calculated assuming no correlation between protein intake and requirement, i.e. risk of deficiency will increase as intakes fall below the recommended daily intake reaching 50 % at an intake = estimated average requirement. (b), The adaptation of metabolic demands to intake removes risk of deficiency in fully adapted individuals until intakes fall to the lower end of the adaptive range, the value of which is unknown but may approach the upper range of the obligatory metabolic demands as indicated by values for the ONL.

of the upper range of the distribution of obligatory MD. At intakes greater than this, the adaptive component of the MD varies directly with intakes and deficiency (negative N balance); this is only likely as a short-term response to a change to a lower intake within the adaptive range (Fig. 5(b)). Risk of protein deficiency would start to become significant at a much lower level than 0·80 g/kg per d, possibly between 0·40 and 0·50 g/kg per d. Most

importantly, as already argued, since diets supplying as little protein as this are highly unlikely to be nutritionally adequate in terms of other nutrients, a dietary recommendation based on the minimum intake for N equilibrium becomes of questionable nutritional significance.

Conclusions

It should be axiomatic that discussions of amino acid and protein requirements occur within a metabolic framework that is both rational and incorporates the observed phenomenology of N and amino acid metabolism and this must include adaptation. From this perspective, the concept of an adaptive MD and the consequences of that for balance regulation is an inescapable conclusion. On the basis of the data shown in Fig. 5(b), for adults satisfying their habitual energy needs on most cereal- or potato-based mixed human diets, intakes will be within the adaptive range. However, it is likely that irrespective of the acceptance or not of adaptive mechanisms, the major factor that has inhibited further discussion is the problem that adaptation poses in terms of public health nutrition policy.

In general, nutrient requirements serve two purposes. One is as a basis for prescription, i.e. advice on safe diets through recommending appropriate dietary intakes. As shown in Fig. 5(b), the adaptive model implies a much lower, but difficult to define, RNI. Formulation of policy in relation to prescriptive matters will inevitably and correctly be most concerned with satisfying the upper range of demands for protein and, where there is uncertainty, including positive margins of error. In this case it is arguably unwise to fully adopt the new model and reduce the RNI, even if agreement could be reached on the likely lower limit of adaptation. Indeed, the MD model does not mean that protein is an unimportant nutrient for the maintenance of human health and well-being, but that indicators other than balance (of N, protein or amino acid) need to be identified. At the moment, notwithstanding the growing experimental evidence for potential benefit of protein intakes considerably greater than the current RNI for bone health (e.g. see New & Millward, 2003), and epidemiological evidence for benefit with regard to hypertension and IHD (see Millward, 1999*b*), such influences are unproven and quantifiable indicators are unlikely to be identified in the short term. This results in a dilemma for those attempting to frame prescriptive dietary guidelines. My own view would be to retain current values as an operational expedient until it becomes possible to quantify the benefits (and any risks) of protein intakes within the adaptive range.

The other purpose of requirement recommendations is to provide the basis for a diagnostic indicator of risk, often within an epidemiological context where population groups rather than individuals are considered. In this case, reference levels of indices used to estimate prevalence of disease states, or deficit risk, are carefully chosen so as to strike an acceptable balance between numbers of false positives and false negatives. The main implication of the adaptive MD model of protein requirements for estimating risk of deficiency (intakes $<$ requirements) is a dramatic reduction in the prevalence of risk for most populations (at least with cereal-based diets) compared with that assessed according to the current model. Risk of deficiency for a population group can be estimated by probability calculations from data describing the distribution of intake and requirement values or estimated approximately as the proportion of the population with intakes below the average requirement value (Beaton 1994, 1999; Institute of Medicine, 2000). This means that group mean intake values must usually considerably exceed the RNI in order that there is an acceptable low prevalence of inadequate intakes (i.e. the group mean intake value should be approximately 2SD of intake above the EAR with SD of intake usually $>$SD of the requirement). In the new model, neither the EAR or RNI can be defined with any certainty, but the values are likely to be very much lower than currently accepted values. Thus, risk of deficiency would also be much lower. As in the prescriptive context, this low risk of deficiency applies only to that of being unable to maintain N balance after full adaptation with otherwise nutritionally adequate diets satisfying the energy demands. Whether such populations enjoy optimal protein-related health in terms of immune function, bone health, growth in height or any other function is a separate issue and needs to be addressed as such. Maintenance of N balance can no longer be used as a surrogate of adequate protein-related health and current lack of quantifiable alternative indicators is no excuse for ignoring the issue of adaptation.

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