

Protein/energy ratios of current diets in developed and developing countries compared with a safe protein/energy ratio: implications for recommended protein and amino acid intakes

D Joe Millward^{1,*} and Alan A Jackson²

¹Centre for Nutrition and Food Safety, School of Biological Sciences, University of Surrey, Guildford, Surrey, GU2 5XH, UK; ²Institute of Human Nutrition, University of Southampton, Southampton SO16 6YD, UK

Submitted 9 October 2002: Accepted 1 October 2003

Abstract

Revised estimates of protein and amino acid requirements are under discussion by the Food and Agriculture Organization (FAO)/World Health Organization (WHO), and have been proposed in a recent report on Dietary Reference Intakes (DRIs) from the USA. The nature and magnitude of these requirements are not entirely resolved, and no consideration has been given to the potential influence of metabolic adaptation on dietary requirements. We have examined the implications of these new values, and of the conceptual metabolic framework in which they are used, for defining the nutritional adequacy of protein intakes in developed and developing countries. We have expressed proposed values for protein requirements in relation to energy requirements, predicted for physical activity levels of 1.5, 1.75 and 2.0 times basal metabolic rate, in order to generate reference ratios for protein energy/total energy (reference P/E ratio) as a function of age, body weight, gender and physical activity level. Proposed values for amino acid requirements have been used to adjust the available digestible P/E ratio of foods and diets for protein quality. Focusing on the diets of UK omnivores and vegetarians and on diets in India, the risk of protein deficiency is evaluated from a comparison of P/E ratios of metabolic requirements with protein-quality-adjusted P/E ratios of intakes. A qualitative and conservative estimate of risk of deficiency is made by comparing the adjusted P/E ratio of the intake with a reference P/E ratio calculated for age, body weight, gender and physical activity according to FAO/WHO/United Nations University. A semi-quantitative estimate of risk of deficiency has also been made by the cut point approach, calculated as the proportion of the intake distribution below the mean P/E ratio of the requirement. Values for the quality-adjusted P/E ratio of the diet range from 0.126 for the UK omnivore diet to 0.054 for a rice-based diet of adults in West Bengal, which is lysine-limited, falling to 0.050 for 1-year-old children. The reference P/E ratio for men and women increases with age, is higher for females than males, is higher for small compared with large adults at any age and decreases with physical activity. Thus if a particular diet is potentially limiting in protein, protein deficiency is most likely in large, elderly sedentary women followed by the adolescent female and least likely in moderately active young children, the opposite of what has usually been assumed. Within the currently accepted framework, the diets do not meet the protein needs of the entire population of the UK, have a significant risk of deficiency throughout India for all except extremely active small adults, and are grossly inadequate for all population groups, apart from physically active young children in West Bengal, regardless of body weight or level of food intake. The lysine limitation of the cereal-based Indian diets is dependent on the choice of lysine requirement values from the published range. We consider that the value selected is too high, because of uncertainties and inconsistencies in the approaches used. A more appropriate choice from the lower end of the range would remove the lysine limitation of cereal-based diets, and reduce some of the perceived risk of deficiency. However, diets remain limited by the amount of digestible protein for many population groups, especially in West Bengal. In the context of risk management, one option would be to accept the current values and the conceptual metabolic framework within which they have been

*Corresponding author: Email D.Millward@surrey.ac.uk

derived. This would have major implications for the supplies of high-quality protein to the developing countries. An alternative option would be to re-evaluate the currently proposed values for the requirements for protein and amino acids. We conclude that the choice of values for the adult lysine requirement should be re-evaluated and that serious consideration should be given to the extent to which adaptive mechanisms might enable the metabolic requirement for protein to be met from current intakes. This will entail a better understanding of the relationships between dietary protein and health.

Keywords
 Protein requirements
 Protein quality
 Protein deficiency
 Food-based dietary guidelines
 Adaptation
 Lysine

Food-based, rather than nutrient-based approaches are increasingly being used in the characterisation of dietary recommendations and guidelines as a means of providing dietary guidance in terms that are understandable to most consumers¹. This shift acknowledges the complex interactions amongst nutrients as classically defined and compounds only recently identified as having potential benefit for the achievement of health. Importantly, food-based guidelines, especially the use of nutrient density, can result in a better definition of nutritional priorities for specific populations. In the context of current debate about the implications of increased recommended intakes of protein and amino acids for nutritional adequacy of diets in developed and developing countries, we have adopted a nutrient density approach to the definition of safe levels of protein intake and estimation of risk of deficiency.

Assessment of the adequacy of both the quantity and quality of protein intakes is an integral part of assessing the adequacy of the human diet. The objective is to avoid a dietary deficiency by ensuring that the intake meets or exceeds the needs of the body, and therefore a comparison is drawn between measures of such needs and intake. In practice, there are considerable difficulties in determining both the needs for protein and the intakes with sufficient reliability to quantify adequacy or risk of deficiency with certainty. Because of this it is necessary to make assumptions about the relationships between the two quantities. As our understanding expands and the behaviour of populations changes, these assumptions have to be revisited. For protein there are two fundamental considerations when judging dietary adequacy. One is qualitative; i.e. the pattern of dietary amino acids in relation to the pattern of amino acid demands of the body. The other is quantitative; the amount of protein that has to be consumed to meet the body's needs, which is determined by the amount of food consumed and its protein density. Because food consumption is primarily determined by energy expenditure, which is a function of basal metabolic rate (BMR) and physical activity level (PAL), and because BMR varies with age, gender and body weight, and physical activity varies with lifestyle and patterns of behaviour, protein consumption is also determined by age, gender, body weight, occupation, lifestyle and patterns of behaviour. Thus these factors need to be considered in any assessment of dietary protein adequacy.

In contrast to this wide variation in intakes, recommendations of appropriate intakes of protein and amino acids, developed from estimates of the metabolic demands of the body for nitrogen and amino acids, are calculated as a constant function of body weight, i.e. expressed as amounts per kg of body weight². Furthermore, with the exception of growth, pregnancy and lactation, protein requirements are assumed not to vary, i.e. to be independent of age, gender, body weight, occupation, lifestyle and patterns of behaviour. This means that assessment of the adequacy of dietary intakes in relation to requirement values must be equally concerned with those factors that determine overall dietary intake as with dietary protein and amino acid composition. The most obvious example of this is the breast-fed infant who satisfies a high demand for protein by consuming large quantities of a very-low-protein food.

In the derivation of recommendations for protein, considerable attention has been given to the composition of the diet, but much less attention to the total diet consumed. The reason for this is that whilst it is accepted that dietary protein utilisation can only be optimal when dietary energy intake satisfies energy demands², it is not assumed that energy expenditure levels and consequent energy intakes influence protein or amino acid requirements. On the contrary, in formulating recommendations for protein, the assumption is made that there is no relationship between the metabolic demands for energy and protein, with the possible exception of sport, although even in this case it is unresolved³. In addition, recommendations for protein do not take into account adaptation, i.e. a variation in requirements for protein with variation in protein intakes, because to date no consensus has been reached about the nature and extent of adaptation^{4–7}. These assumptions might not be warranted. As a consequence, it is not possible to answer the simple question: Will a particular dietary composition allow adequate protein intakes for a given population group?

When the primary concern is for the adequacy of a diet, any concern about the adequacy of dietary protein (or any other nutrient) has to start from the presumption that energy balance has been achieved. Thus, the energy needs of the population can be assessed, on the assumption that energy balance has been achieved⁸. This can be used as the basis for expressing nutrient needs in terms of nutrient density, i.e. the nutrient to energy ratio or, for protein, the

ratio of protein energy to total energy (P/E ratio). If the dietary intake is also expressed in terms of a P/E ratio, then intake and requirements can be compared taking into account those factors that influence energy requirements, i.e. age, gender, body weight, lifestyle and patterns of behaviour. Potential dietary protein deficiency can then be identified when a particular diet, consumed to meet energy needs, fails to meet the protein need for any specific population group. The ability to evaluate specific groups at special risk, rather than whole populations, is an obvious advantage of this approach. A second advantage relates to advice to the public about their dietary needs, when recommendations for patterns of consumption are better expressed as food-based rather than nutrient-based guidelines. It is only by expressing nutrient requirements as a nutrient to energy ratio that food-based dietary guidelines can be readily developed. However, one consequence of this approach is the confusing, counter-intuitive implications, which have to be recognised and understood if the approach is to be applied effectively. Thus, as indicated above, the very high energy requirements during infancy and childhood, which decrease with age at a greater rate than the fall with age in the protein requirement, means that the P/E ratio of the requirements for infants is low and increases with age. Furthermore, the counter-intuitive implication of this is that a diet that can meet both the energy and protein needs of the infant may satisfy the energy needs of older children or adults, but may fail to meet their needs for protein at the level of consumption required to meet needs for energy. A general note of these important points was made by the Food and Agriculture Organization (FAO)/World Health Organization (WHO)/United Nations University (UNU)², but they were not explored in any detail. Indeed, although the P/E ratio has been used widely to express the protein content of the diet, it has not generally been used to characterise the safe level of protein consumption required to meet the metabolic demands of the body.

FAO/WHO/UNU is currently re-examining recommendations for protein and amino acid intakes, and revised values have recently been published in a report from the USA on Dietary Reference Intakes (DRIs)⁹. Our intention is to explore how these recommendations relate to diets as consumed by population groups in developed and developing countries by comparing P/E ratios of requirements and intakes. We are particularly concerned with exploring which population groups within any country are most likely to be at risk of protein deficiency for any estimate of the requirement. It is especially important to determine whether there are issues of concern for populations where food intake, and hence protein consumption, has fallen as a result of significant reductions in physical activity patterns, and whether this carries any special risk for populations that consume diets of marginal quality. We also explore whether risk of protein deficiency calculated from current estimates and

requirement models for protein and amino acids requirements are consistent with current estimations of the adequacy or inadequacy of diets. This is important in the context of adaptation because the relevance of adaptation becomes especially important for populations where risk of protein deficiency appears high when current approaches are adopted.

Methods

To assess the risk of dietary protein deficiency within a population group in the context of nutrient densities, it is necessary to compare the P/E ratio of the dietary intake with a P/E ratio that is considered safe based on the need to satisfy the metabolic requirements. We have defined and estimated a *reference P/E ratio*, which is taken to represent a safe pattern of consumption, and estimated the *mean P/E ratio of the requirement* as the basis for assessing the risk of deficiency within a population group.

Requirement

Protein requirements

Values for the protein requirements have been derived from a meta-analysis of all available nitrogen-balance data¹⁰ and are similar to estimates of mean requirements and variance used in the development of the report on DRIs in the USA⁹. This is the same database that will form the basis of recommendation for protein requirements by FAO/WHO/UNU in their current review.

Energy requirements

Values for energy requirements have been calculated from estimates of energy expenditure predicted from age- and body-weight-specific predicted values for BMR² for both men and women using the Schofield equations¹¹, and physical activity levels (PAL values) of 1.5, 1.75 and 2.00, which define light, moderate and heavy activity.

Intakes

In the following analysis, the intake data are approximate and draw on food intake and food supply data expressed as P/E ratios of intakes calculated from the protein and energy contents of foods as available or as consumed in diets in both developed and developing countries. Since our major concern is the P/E ratio of the food supply as a measure of its composition, reported values can be validated to some extent by comparison with the P/E ratio of specific cereals and legumes. For the purpose of this exercise we have used the 1990 UK adult nutrition survey as analysed by Jackson and Margetts¹², which identified data on intakes of omnivores and vegetarians (non meat-eaters), and data for the average Indian diet and for West Bengal as reported by Pellett¹³. Approximate values for the variability of the intake have been used based on the reported variance of the P/E ratio of

the UK intakes¹² and this value has been assumed to apply to the variability in the consumption of the two Indian diets.

Adjustments of dietary protein sources for their quality

The available dietary protein reflects the amount and protein density of food intake, and protein quality in terms of both digestibility and biological value (BV). BV (retained nitrogen/absorbed nitrogen) is a function of the amino acid score, calculated from the dietary protein amino acid pattern in relation to the amino acid pattern of the metabolic demands of the body¹⁴.

The majority of the population of the world live in developing countries and plant proteins constitute the major source of proteins, with cereals being predominant. Of these, wheat (43%), rice (39%) and maize (12%) account for the main part¹⁵. It is widely perceived that plant protein sources differ from animal sources in terms of their amino acid composition, although these differences may be of minor importance especially when mixtures of plant protein sources are being consumed. However, there are differences in terms of digestibility, which may be more important, especially in the presence of anti-nutritional factors that influence digestibility adversely. Thus decisions need to be made about both digestibility and amino acid score.

Digestibility

The important issue of digestibility relates to the plant cell wall, as once plant cell wall constituents are removed the inherent digestibility of plant proteins may be indistinguishable from that of animal proteins. Thus, food sources with high digestibility (>95%, eggs/milk/meat) also include wheat gluten, wheat flour and soy protein isolate¹⁴. This contrasts with the lower digestibility for whole-grain cereals, peas, polished rice, soy flour, chick-pea and pea protein isolates of 80–90%, or for whole millet, beans, breakfast cereals and mixed diets in developing countries of 50–80%¹⁴. These differences reflect particularly resistant plant cell walls (millet and sorghum), anti-nutritional factors (beans) or processing and heat treatment (breakfast cereals). Much of the data derive from animal studies, which give results similar to studies in adults or young children generally^{16–18}. In some cases (e.g. sorghum) poor digestibility can be improved to some extent by fermentation and processing. Clearly, for some plant protein sources, digestibility can reduce nutritional value, but on the basis of these data a value of 80% can be used as an overall guide for diets based on the main cereal sources, recognising that some diets may be worse.

We have only a limited appreciation of the complex handling of amino acids and nitrogen compounds between the small and the large intestine, and this has important implications for the concept of digestibility. In practice, the determination of digestibility is based on

the nitrogen appearing at the terminal ileum (ileal digestibility) or in stools (faecal digestibility). For ileal digestibility, loss of amino acids in the colon but retention of the nitrogen as ammonia means that true digestibility is overestimated for some amino acids^{19,20}. For plant protein sources that are poorly digested in the upper intestine and which result in a significant nitrogen and indispensable amino acid (IAA) flow into the colon, the measured ileal digestibility would indicate poor digestibility. On the other hand, the demonstration of *de novo* synthesis of IAA during bacterial nitrogen metabolism and urea salvage, with absorption from the lower gut^{21–23}, means that the potential exists for colonic nitrogen metabolism to add some IAA to the dietary supply, thereby improving apparent digestibility. These two considerations make it difficult to interpret the practical implications of conventional measures of digestibility^{24,25}. However, at present, lack of adequate information and limitations in our understanding mean that it is not possible to determine how they should be factored into any calculations. Therefore, for the present purpose, the assumption has been made that overall nitrogen digestibility is the weighted mean of 95% and 80% for animal and plant protein sources, respectively. Clarification of this uncertainty is an important area for further work.

Amino acid composition and BV

BV can be directly assessed in nitrogen-balance trials or predicted from amino acid score, i.e. its amino acid composition in relation to a reference pattern. In adults the direct measurement of the BV of proteins has not proved useful. The apparent efficiency of utilisation of all proteins including egg, milk and meat measured in nitrogen-balance studies in adults is low, contrary to what would be predicted from animal studies²⁶. A recent meta-analysis of human balance studies²⁷ indicates a mean apparent slope of 0.46 for high-quality animal protein (retained nitrogen/absorbed nitrogen). As these proteins are not limited by their amino acid content, a slope close to the value of 1 would be expected. Furthermore, in the same meta-analysis, the slopes were similar to that for high-quality animal protein for vegetable and mixed protein sources, 0.47 and 0.48 respectively. As discussed below, we consider that these unexpected findings can best be explained by metabolic adaptation. However, because of these difficulties in measuring BV directly, estimates are predicted from the amino acid composition of protein. These estimates have been based on a scoring pattern (mg amino acid/mg protein) which is calculated from the requirements for IAA and for protein. It is assumed that requirements for both protein and amino acids vary with age, so that the predicted BV of a dietary protein will vary with age, being lower when consumed by infants than adults because of the higher amino acid requirements of infants.

Age-specific amino acid scoring patterns

FAO/WHO is in the process of revising the IAA requirements and recommended safe levels of consumption for dietary protein given in the 1985 report². Recently estimates have been published in the USA as a report on DRIs⁹. In the DRI report it is suggested for adults that the mean dietary requirement for protein is about 10% higher than given by FAO/WHO/UNU in the 1985 report², but with the same coefficient of variation (CV; 12%). For young children we have derived a new set of requirement values based upon a factorial analysis²⁸, which are slightly lower than those given by FAO/WHO/UNU in the 1985 report². Here, we have used these recent estimates²⁸ in which protein requirements range from $0.88 \text{ g kg}^{-1} \text{ day}^{-1}$ at 1 year of age to $0.66 \text{ g kg}^{-1} \text{ day}^{-1}$ in adults.

For amino acids, the US DRI report⁹ has adopted higher dietary requirements for most IAA than the values recommended by FAO/WHO/UNU in the 1985 report². The major change of practical importance involves lysine, the most important limiting amino acid in cereal-based diets. FAO/WHO/UNU is likely to recommend an increase in the requirement for lysine from 12 to $30 \text{ mg kg}^{-1} \text{ day}^{-1}$, and we have used this value in the present analysis. This is similar to the value of $31 \text{ mg kg}^{-1} \text{ day}^{-1}$ for the requirement for lysine identified in the DRI report⁹. The justification for a change derives from a reanalysis of the original nitrogen-balance data^{26,27} and stable isotope studies²⁹⁻³⁶. While all accept the need for a revision of the value for the lysine requirements, there is considerable uncertainty and controversy about the best estimate, as discussed below. For infants and children the amino acid requirement values are calculated factorially based on a value for maintenance plus a value for growth. We have adopted the same maintenance value for children as the adult value, i.e. $0.66 \text{ g kg}^{-1} \text{ day}^{-1}$. The value for growth is based on the assumed dietary protein needs for growth and the IAA composition of body protein (e.g. 73 mg lysine/g protein)²⁸. Values for lysine requirements calculated in this way, and how they translate to protein requirements at different ages, are shown in Fig. 1.

Overall value of dietary protein quality: the PDCAAS-corrected P/E ratio of foods and diets

Variation in digestibility will reduce the amino acids available to metabolism and any amino acid inadequacy predicted from the amino acid score will lower the efficiency of utilisation of food protein by the body, so that the available P/E ratio of the diet will be lower than the P/E ratio of food as consumed. Thus the nutritional value of dietary protein is predicted from a protein-digestibility-corrected amino acid score: the PDCAAS value¹⁴. The PDCAAS is the product of digestibility and amino acid score, and has an equivalence to net protein utilisation. Thus, the amount of available protein in relation to the energy content of a food protein or diet is predicted as PDCAAS-adjusted P/E ratio. This is in effect the value

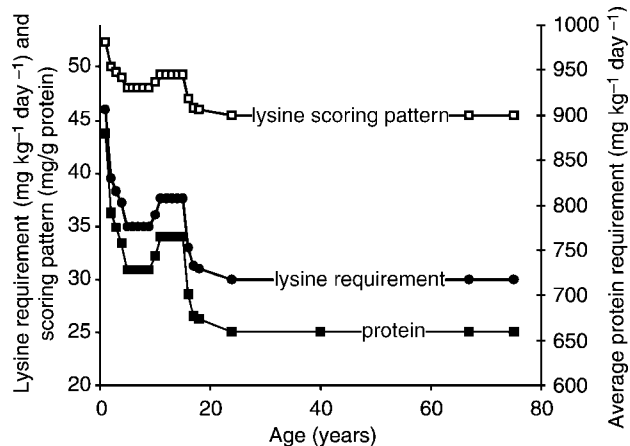


Fig. 1 Lysine and protein requirement values and the derived lysine scoring pattern used to judge age-related changes in biological values of diets. Values calculated from factorial estimates of lysine and protein requirements calculated according to Dewey *et al.*²⁸ on the basis of $30 \text{ mg lysine kg}^{-1} \text{ day}^{-1}$ and $660 \text{ mg protein kg}^{-1} \text{ day}^{-1}$ for maintenance requirements of lysine and protein, protein needs for growth using smoothed values for tissue protein accretion calculated from the body composition data of Butte *et al.*³⁷ and Ellis *et al.*³⁸ adjusted for an assumed dietary efficiency of 61% and a tissue lysine content of 73 mg/g protein

introduced many years ago by Platt *et al.*³⁹ defined as nDPCals%, and provides a measure of the ability of a food to supply utilisable protein.

Assessing nutritional adequacy of dietary intakes*Dietary assessment and calculation of risk of deficiency*

Beaton has discussed the procedures involved in the assessment of risk of dietary protein deficiency within a population group and derivation of reference P/E ratios^{2,41,42}. His ideas have been incorporated into the recently published report *Dietary Reference Intakes: Application in Dietary Assessment*⁴⁰. Such an exercise is particularly difficult when dealing with nutrient density and needs to be conducted with care. For a complete assessment, information is required about appropriate values for both requirements and intakes of protein and energy, and the nature and extent of both the within-individual and between-individual variation. Information is also required about the extent of any correlations between (1) intakes and requirements for energy, (2) intakes and requirements for protein and (3) energy and protein requirements.

When the data are available to enable the generation of representative distributions for both intakes and requirements, it is possible to use a probability approach and to calculate the proportion of the population who is deficient from the distribution curves. However, representative data on intakes and requirements are generally not available within the same population groups, and therefore it is necessary to develop approaches within which reasonable assumptions can be made about the variables of importance and that can be used to estimate risk of deficiency, even if there is some imprecision.

In all of the approaches that have been used for estimating risk of protein deficiency, it has been assumed that there is no correlation between the intake of protein and protein requirements. Furthermore, the conceptual metabolic framework that has been accepted for estimating risk of deficiency does not recognise the possibility that metabolic adaptation plays any part. Therefore metabolic adaptation is not considered to operate or exert any influence on the relationship between requirement and intake. Here we discuss and adopt different approaches to evaluation of the relationship between the P/E ratio of the requirements and the P/E ratio of the dietary intake.

Derivation of a reference P/E ratio to judge adequacy of population intakes

Unfortunately, there has been no clear agreement on the most appropriate way or even the feasibility of deriving a reference P/E ratio, which would indicate the safe level of intake for a population group that is conceptually similar to the Reference Nutrient Intake (RNI) as an indication of the safe level of intake for an individual. The data needed to enable a detailed analysis of the risk of deficiency for all ages, body weights and both genders in the developing world are not available. Therefore, a pragmatic approach has been adopted, in effect by inverting the question and asking: Given existing patterns of physical activity, and therefore energy expenditure (and intake), what P/E ratio is necessary to meet the protein requirements? To address this question it is necessary to generate a set of P/E ratios of requirements for varying levels of energy expenditure, and from these generate a set of reference P/E ratios that would represent a safe intake for that population group. The reference P/E ratios can then be compared with the protein-quality-corrected P/E ratios of diets as consumed. The first step, generating P/E ratios of requirements, is not easy.

In the 1985 FAO/WHO/UNU report (Appendix 9A)², in the context of assessing *individual* diets, a reference (i.e. safe) P/E ratio was calculated from mean protein and energy requirements and their variability. The value calculated by this formula approximates:

$$\frac{\text{EAR}_{\text{protein}} + 3\text{SD } \text{EAR}_{\text{protein}}}{\text{EAR}_{\text{energy}}},$$

where EAR is the Estimated Average/median Requirement for protein or energy and SD is the standard deviation. Furthermore, in that report it was argued that to assess the suitability of diets consumed by *populations*, the variance of *dietary* P/E ratios needed to be taken into account, which means that the appropriate reference P/E ratio should be further increased to a value approximating to:

$$\frac{\text{EAR}_{\text{protein}} + 4\text{SD } \text{EAR}_{\text{protein}}}{\text{EAR}_{\text{energy}}}.$$

The extent to which the variance of dietary P/E ratios should be taken into account has to be looked at critically.

In subsequent articles, Beaton^{41,42} argued that the mean protein intake of a population which would be associated with low risk of deficiency is usually considerably higher than $\text{EAR}_{\text{protein}} + 2\text{SD } \text{EAR}_{\text{protein}}$, i.e. the RNI or safe level of intake as in FAO/WHO/UNU 1985², and equal to about 2 SD of the protein *intake* above the EAR ($\text{EAR}_{\text{protein}} + 2\text{SD } \text{intake}_{\text{protein}}$). Thus the magnitude of the mean intake of a population which would be considered safe when related to the RNI would depend on the relative magnitude of the variances of both *intake* and *requirement*, with the SD of the former usually considerably larger than that of the latter. Most of the worked examples that have been reported have been based on the protein requirements and intakes of adults in the USA, and do show that the mean intake of the population which is safe, when calculated in this way, is indeed much greater than the RNI⁴². Other examples can be used. In the 1990 UK adult nutrition survey, as analysed for protein intakes by Jackson and Margetts¹², the SD of $\text{intake}_{\text{protein}}$ was approximately $0.22 \text{ g kg}^{-1} \text{ day}^{-1}$ (0.24 and $0.20 \text{ g kg}^{-1} \text{ day}^{-1}$ for men and women, respectively), which would indicate a safe mean intake by the population of $1.1 \text{ g kg}^{-1} \text{ day}^{-1}$ (as $\text{EAR}_{\text{protein}} + 2\text{SD } \text{intake}_{\text{protein}}$, where $\text{EAR}_{\text{protein}} = 0.66 \text{ g protein kg}^{-1} \text{ day}^{-1}$). This would be equivalent to $\text{EAR}_{\text{protein}} + 6\text{SD } \text{EAR}_{\text{protein}}$. In the most recent UK adult nutrition survey⁴³, $\text{intake}_{\text{protein}}$ was $88 \pm 32 \text{ g day}^{-1}$ (CV 33%) for men and $64 \pm 17 \text{ g day}^{-1}$ (CV 26%) for women (mean $\pm 1\text{SD}$). Assuming a mean body weight of 74 kg for men and 60 kg for women, the SD for $\text{intake}_{\text{protein}}$ would be $0.44 \text{ g protein kg}^{-1} \text{ day}^{-1}$ for men and $0.28 \text{ g protein kg}^{-1} \text{ day}^{-1}$ for women. This would approximate to safe mean intakes for the population of between 1.22 and $1.54 \text{ g kg}^{-1} \text{ day}^{-1}$ (as $\text{EAR}_{\text{protein}} + 2\text{SD } \text{intake}_{\text{protein}}$, where $\text{EAR}_{\text{protein}} = 0.66 \text{ g protein kg}^{-1} \text{ day}^{-1}$). However, the intakes of protein by normal men ($1.19 \text{ g kg}^{-1} \text{ day}^{-1}$) and women ($1.07 \text{ g kg}^{-1} \text{ day}^{-1}$) in the UK are not associated with any obvious evidence of protein deficiency. Hence, using this approach, there is a clear discrepancy between the estimate of a safe level of protein intake and the observed intake in a population that is apparently well-nourished.

In further discussion about safe levels of the P/E ratio, Beaton used simulated data derived from published values for energy and protein requirements and intakes of young US adults assumed to exhibit the same moderate levels of energy expenditure. This analysis suggested that, for young males, the population mean P/E ratio of the intake associated with negligible risk of deficiency was 0.13. If one were to accept this value of 0.13 for the P/E ratio, it would be equivalent to using $\text{EAR}_{\text{protein}} + 8\text{SD } \text{EAR}_{\text{protein}}$ to calculate a safe P/E ratio for the mean intake of a population. This value is considerably higher than the average P/E ratio of intakes for many populations (e.g. the average P/E ratio for India is 0.11)¹³, before any adjustment for protein quality. A value of 0.13 for the P/E ratio can be compared with a value of P/E ratio of

0.093 for the 95th percentile of the simulated distribution of P/E for the metabolic requirement; 0.075 for $RNI_{\text{protein}}/EAR_{\text{energy}}$ or 0.06 for $EAR_{\text{protein}}/EAR_{\text{energy}}$.

It has to be recognised that the robustness of this kind of analysis is heavily dependent on the data available for analysis, and these data are extremely limited and unlikely to be available for most populations. Furthermore, the model carries important limitations that may result in overestimation of safe values.

First, it is usually assumed that variation in intakes can be interpreted simply in terms of individuals eating either more or less than their requirement (with it being assumed that intake and requirement are not correlated). In all studies of food intake, variable underreporting accounts for substantial variation⁴⁰. Furthermore, energy intake is to a large extent regulated by appetite and intake varies to match changes in activity and energy expenditure. For protein and those nutrients supplied by major energy sources in the diet, this means that variation in energy expenditure, and hence food energy intake, will constitute an important component of variation in nutrient intake, since it can be assumed that for most population groups dietary composition does not vary to a large extent with the level of energy expenditure. Variability in protein consumption due to different levels of energy expenditure, and hence food energy intake, would increase the overall SD of the intake_{protein}, which would spuriously overestimate a safe mean population intake calculated as $EAR_{\text{protein}} + 2SD \text{ intake}_{\text{protein}}$.

Second, in the present circumstances the intention is to compare measures of the protein requirements with protein intakes both for several different populations consuming quite different diets and for different populations in terms of age, gender and physical activity patterns consuming the same diet. Therefore, given that the SD of the intake_{protein} may differ for each population group, the identification of a safe mean population intake, calculated as $EAR_{\text{protein}} + 2SD \text{ intake}_{\text{protein}}$, could differ for overall populations and for subgroups within the population.

In terms of the present exercise, it follows from this that a global comparison of intakes and requirements for men and women at all ages and body weights, involving a single formula, cannot be done with any certainty and a judgement needs to be made about a suitable calculation of a reference P/E ratio from protein and energy requirements at different levels of physical activity in terms of its likely under- or overestimation of risk of deficiency. On the basis of the above discussion that $EAR_{\text{protein}} + 2SD \text{ intake}_{\text{protein}}$ may overestimate the safe mean population intake while the safe reference P/E ratio as derived in the 1985 FAO/WHO/UNU report² (i.e. equivalent to $(EAR_{\text{protein}} + 3SD \text{ EAR}_{\text{protein}})/EAR_{\text{energy}}$) may underestimate the safe mean population intake, it follows that use of the latter value will result in a relatively conservative assessment of risk. Therefore, in the

combined approach which we develop below, we have derived a reference P/E ratio that might be taken as representing a safe intake, using the formula given in the 1985 FAO/WHO/UNU report², i.e. approximating to

$$\frac{EAR_{\text{protein}} + 3SD \text{ EAR}_{\text{protein}}}{EAR_{\text{energy}}},$$

from values for EAR_{protein} and EAR_{energy} , with an assumed variance of 12% for both protein and energy requirements. We have further assumed a low level of correlation between requirements for protein and energy (r is assumed to be 0.1). While this derivation is considerably lower than the value for the mean intake of any population identified as a reference safe intake in the past – which, as discussed above, may be an overestimate of the safe intake, it may still be lower than the safe intake. Therefore, in adopting it here we are assuming that any indications of likely protein deficiency will involve conservative judgements of dietary adequacy.

Estimate of risk of deficiency: EAR cut point method, based on the mean P/E ratio of requirement

An alternative to full probability calculations of the extent of deficiency for a population from distributions of intakes and requirements is to utilise the fact that the extent of deficiency for a population is approximated by the proportion of the population with intake_{protein} below EAR_{protein} . This simplified approach is known as the EAR cut point method^{40,42}. Thus, assuming Beaton's proposition that percentage risk of an inadequate protein intake for an individual in a population is the proportion of the intake distribution falling below the mean requirement value, we have estimated where the mean requirement (EAR_{protein}) lies on the intake distribution. If EAR_{protein} lies at $-1SD$ of the intake_{protein} distribution, then 16.5% of the population is at risk of deficiency; if EAR_{protein} lies at $-2SD$ of the intake_{protein} distribution, then 2.5% of the population is at risk of deficiency. Thus, for any population with a requirement of x and an intake of y , nutritional adequacy occurs when $y - 2SD \text{ } y \geq x$: that is, when $-2SD$ (Z -score of -2) of the intake_{protein} distribution is greater than EAR_{protein} . Thus, for nutritional adequacy:

$$\text{mean intake}_{\text{protein}} - 2SD \text{ intake}_{\text{protein}} \geq EAR_{\text{protein}}.$$

Using this approach we have made more precise (although still very approximate) estimates of the risk of deficiency for selected groups. In the derivation below, the assessment of risk has been estimated as the proportion of the population with a P/E ratio for the intake that is less than the P/E ratio for the mean protein requirement ($EAR_{\text{protein}}/EAR_{\text{energy}}$), by using the NORMDIST function within Excel to determine the cumulative distribution of a value equal to the mean P/E ratio of the requirement within the distribution of P/E intakes given by the mean and SD values for the P/E ratio of the diet.

A combined two-step approach to determination of dietary adequacy

For the present exercise, we have adopted a two-step approach involving aspects of the approaches used to determine requirements and intakes.

- *Step 1:* In order to identify at-risk population subgroups we have calculated a safe reference P/E ratio as derived in the 1985 FAO/WHO/UNU report² (i.e. approximating to $(EAR_{\text{protein}} + 3SD \text{ } EAR_{\text{protein}})/EAR_{\text{energy}}$), recognising its limitations. We define this as the *reference P/E ratio*. This formula has been used to calculate a reference P/E ratio for men and women at all ages, body weights and levels of physical activity. The reference P/E ratio has been compared with the protein-quality-adjusted P/E ratio of actual intakes.
- *Step 2:* The EAR cut point method has been used to estimate the risk of deficiency for selected population groups identified in step 1, based on the *mean P/E ratio of the requirement* (as $EAR_{\text{protein}}/EAR_{\text{energy}}$) and the mean and SD values for the P/E ratio of the dietary intake.

Neither approach makes any allowance for metabolic adaptation, i.e. both approaches assume that there is no interdependence of dietary protein intake and the metabolic requirement for protein.

Results

P/E ratio of foods and diets

Table 1 shows the adjusted P/E ratios for plant and animal proteins and for some diets based on their reported composition adjusted for the proposed adult lysine requirement and assumed values for digestibility. Wheat and maize exhibit the lowest PDCAAS value and the reduced BV of cereals explains the low BV of many Indian diets. Amino acid scores for cereals range from 57% in wheat to 95% in the most improved strains of maize (mz:o2s2 variety). Soy meets the requirement for lysine, and also meets the likely proposal for sulphur amino acids. The adjusted P/E ratios are shown in the final column of Table 1 and Fig. 2. The considerable differences between

Table 1 PDCAAS-adjusted P/E ratios of animal and plant food sources and diets

Food or diet	P/E ratio	Lysine content (mg/g protein)	Lysine score*	Digestibility†	PDCAAS	PDCAAS-adjusted P/E ratio
Beef	0.66	91	213	100	100	0.660
Egg	0.34	70	283	100	100	0.340
Cow's milk	0.19	78	233	100	100	0.194
Breast milk	0.060	69	283	100	100	0.060
Soy	0.388	65	100	90	90	0.349
Wheat	0.166	26	57	95	54	0.089
Maize	0.135	29	64	82	52	0.071
mz:o2	0.140	40	88	80	70	0.098
mz:o2s2	0.140	43	95	80	76	0.106
Potatoes	0.097	54	100	82	82	0.079
Rice	0.072	36	79	82	65	0.047
Yam	0.061	42	91	80	73	0.045
Cassava	0.034	32	71	80	57	0.019
Cassava–soy: 90 : 10 energy mix	0.069	51	100	86	86	0.059
<i>Food balance data</i>						
USA‡	0.121	67	100	90	90	0.109
UK‡	0.110	64	100	89	89	0.097
Tunisia‡	0.109	43	95	83	79	0.086
Egypt‡	0.094	40	88	82	73	0.068
Brazil‡	0.093	60	100	86	86	0.080
Nepal‡	0.102	43	94	82	77	0.079
Bangladesh‡	0.084	44	97	82	80	0.067
Sierra Leone‡	0.080	51	100	83	83	0.067
<i>Food intake data</i>						
UK omnivores§	0.142	65	100	89	89	0.126
UK vegetarians§	0.127	53	100	81	81	0.102
Indian average§	0.111	39	86	80	69	0.076
Tamil Nadu§	0.097	44	97	80	77	0.075
West Bengal§	0.088	35	77	80	62	0.054
Bangalore: well-nourished¶	0.110	53	100	83	83	0.091
Bangalore: undernourished¶	0.080	49	100	83	83	0.066
Indian mean + legumes: 0.92 : 0.078 energy mix	0.132	45	100	80	80	0.106
West Bengal + legumes: 0.90 : 0.10 energy mix	0.119	45	100	80	80	0.095

PDCAAS – protein-digestibility-corrected amino acid score; P/E – protein/energy.

* Based on an adult reference value for lysine of 45 mg/g protein (see text).

† Assumes overall nitrogen digestibility is the weighted mean of 95% and 80% for animal and plant protein sources, respectively. For Bangalore data, % animal protein intakes calculated from lysine intake data assuming that animal and plant proteins contain lysine at 80 and 35 mg per g, respectively.

‡ Food and Agriculture Organization food balance sheets 1961–1992, as reported by Pellett¹³.

§ Intake data from Jackson and Margetts¹² with lysine concentration as in Pellett¹³.

¶ Measured intakes in a dietary survey by AV Kurpad (personal communication, 2001).

the protein content of wheat and maize compared with rice, or more especially yam and cassava, lead to quite marked differences in the adjusted P/E ratios for the different staples. The overall range for individual food components is from 0.66 for beef to 0.019 for cassava. As is well known, consuming legumes with cereals improves overall nutritional value. For example, in Fig. 2 the effect is shown of mixing legumes and cassava where, with only 10% of the energy coming from soy, the P/E ratio of the mix is doubled.

For diets (Fig. 2), the adjusted P/E ratios range from 0.126 for the UK omnivore diet to 0.054 for the West Bengal diet, in which protein derives from rice (90%), pulses (6%) and animal sources, mainly milk (1.5%)¹³. This low adjusted P/E ratio is similar to that of the diet consumed by adults with a low body mass index in slums in Bangalore (AV Kurpad, personal communication, 2001). The higher P/E ratio of wheat compared with all other cereals means that wheat-based diets supply more utilisable protein than rice-based diets. Figure 3 shows the effect of the addition of legumes (soy) to improve

the BV of the average Indian diet or that of West Bengal. The dietary lysine requirement for adults (45 mg/g dietary protein) would be met by the addition of 7.8 or 10% of legume energy, respectively, to the two diets. This would increase the available P/E ratio to 0.106 and 0.095, i.e. close to that of the UK vegetarian diet.

Reference P/E ratio

Table 2 shows the reference P/E ratio (approximating to $(EAR_{protein} + 3SD \text{ } EAR_{protein})/EAR_{energy}$), for selected ages from infancy to old age for males and females at two different adult body weights. The $EAR_{protein}$ is defined as a constant function of body weight in adults. For infants and children, $EAR_{protein}$ is defined as a function of body weight plus an increment to allow for the needs for growth. The change with age is very small. In contrast, EAR_{energy} per kg body weight varies markedly. This is a consequence of the variation in BMR and variation in the level of energy expenditure at different ages and in different states. Thus the reference P/E ratio increases with age, is higher for females than males, is higher for small compared with large adults at any age, and decreases with physical activity. Hence, as shown in Table 2, the reference P/E ratio is lower in infants and children than in adults, 16% lower in adults weighing 50 kg than in adults weighing 70 kg, 14% lower in young and middle-aged men than in women at the same age or weight, and 8% lower in older men than in women. Thus, on the basis that the reference P/E ratio represents a

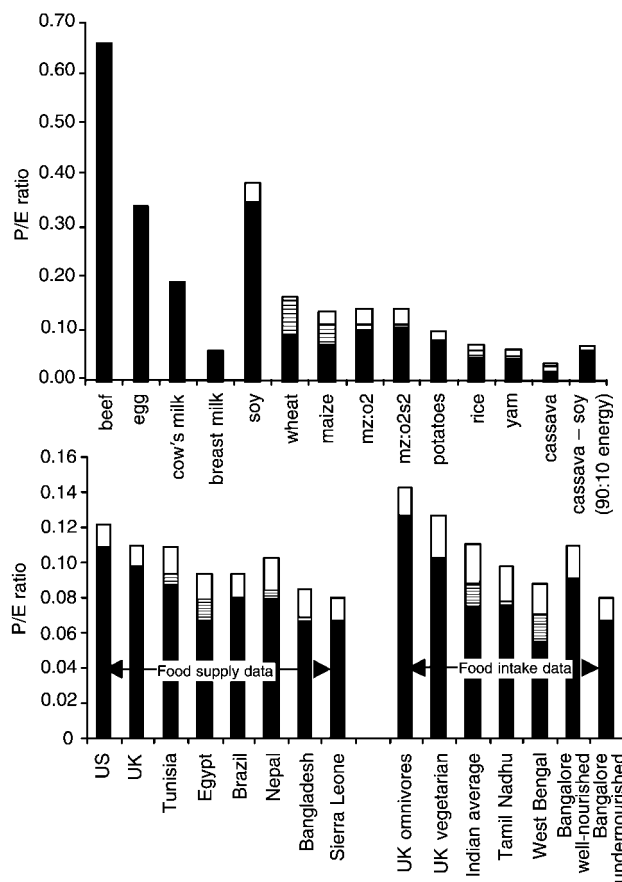


Fig. 2 Protein/energy (P/E) ratio of dietary proteins (top) and diets (bottom). Values shown represent gross P/E ratio (overall bar heights), PDCAAS-corrected values (■) after adjusting for biological value (▨) and digestibility (□). Source of data as in Table 1. PDCAAS, protein-digestibility-corrected amino acid score

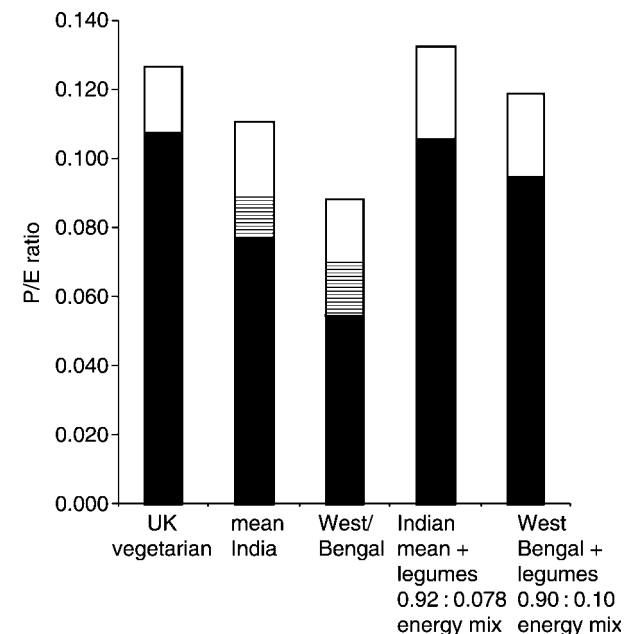


Fig. 3 Impact of dietary supplementation with legumes on the protein/energy (P/E) ratio of dietary proteins. Values shown represent gross P/E ratio (overall bar heights), PDCAAS-corrected values (■) after adjusting for biological value (▨) and digestibility (□). Supplementation is at a level that will increase the lysine/protein ratio of the diet to 45 mg/g protein (the adult lysine requirement) and involves addition of 7.8 or 10% of legume energy from soy to the mean Indian and West Bengal diets, respectively. PDCAAS, protein-digestibility-corrected amino acid score

Table 2 Reference protein/energy (P/E) ratios for selected age groups of males and females

		Average protein requirements*						Males			Females				
		Males and females		Energy requirements†		Reference P/E ratio‡		Energy requirements†			Reference P/E ratio‡				
Age (years)	Deposition (mg kg ⁻¹)	Growth requirements (e = 61%) (mg kg ⁻¹)	Maintenance (mg kg ⁻¹)	Total (mg kg ⁻¹)	Protein calories (kcal kg ⁻¹)	PAL	PAL	PAL	PAL	PAL	PAL	PAL	PAL		
														1.5	1.75
1	131	215	660	875	3.5	77.0	88.1	0.058	0.053	–	82.7	93.5	0.058	0.052	–
5	55	90	660	750	3.0	67.5	77.2	0.052	0.052	–	71.7	81.0	0.056	0.049	–
<i>Adults at 70 kg body weight</i>															
18–29			660	660	2.64	37.5	43.7	0.097	0.083	0.072	32.6	38.1	0.111	0.095	0.083
30–59			660	660	2.64	36.0	42.0	0.101	0.086	0.076	30.6	35.7	0.118	0.102	0.089
60–74			660	660	2.64	33.2	38.7	0.109	0.094	0.082	28.6	33.4	0.127	0.109	0.095
>75			660	660	2.64	30.2	35.2	0.12	0.103	0.09	28.1	32.8	0.129	0.11	0.097
<i>Adults at 50 kg body weight</i>															
18–29			660	660	2.64	43.4	50.6	0.083	0.072	0.063	36.8	42.9	0.098	0.084	0.074
30–59			660	660	2.64	43.4	50.7	0.083	0.071	0.063	37.8	44.1	0.096	0.082	0.072
60–74			660	660	2.64	39.3	45.8	0.092	0.079	0.069	34.5	40.3	0.105	0.090	0.079
>75			660	660	2.64	37.2	43.4	0.097	0.083	0.073	33.5	39.0	0.108	0.093	0.081

PAL – physical activity level (PAL = 1.5, light; PAL = 1.75, moderate; PAL = 2.0, heavy).

* Protein requirement values for infants and children calculated from factorial estimates of requirements calculated according to Dewey *et al.*²⁸ on the basis of maintenance requirements⁹ of 660 mg kg⁻¹ day⁻¹ and protein needs for growth adjusted for an assumed dietary efficiency of 61%. Protein calories = protein requirement (g) × Atwater factor, 4 cal g⁻¹.

† Energy requirements calculated on the basis of National Center for Health Statistics/World Health Organization (WHO) median weights-for-age as reported by Torun *et al.*⁴⁴ using Schofield equations for basal metabolic rate adjusted for PAL values of 1.5, 1.75 and 2.0¹¹.

‡ Reference P/E requirement values calculated according to the formula given by WHO/Food and Agriculture Organization/United Nations University² (Annexe 9A), which is an estimate of the reference P/E requirement value for an individual, and equates to about (EAR_{protein} + 3SD EAR_{protein}) / EAR_{energy}. It is likely to underestimate the actual safe P/E requirement values for a population (see text).

safe or 'desirable' P/E ratio that has to be provided by the diet (recognising the caveats above), a sedentary elderly women who weighed 70 kg would require food with more than twice the protein concentration relative to energy compared with the diet needed by very young children, and 5–8% more than that required by elderly men of the same weight. The difference in the 'desirable' P/E ratio of the diet between adult men and women would be more marked if we assumed that PAL values for any particular activity category are lower for women than men, as is assumed in calculating EAR_{energy} for children and adolescents⁴⁴. We have not done this here.

Comparison of reference P/E ratio with P/E ratio of diets

To further explore age-related differences in the reference P/E ratio we have calculated values for population groups of all ages from 1 to 75 years, at three levels of physical activity with adult weights representative of developed countries, i.e. 57 kg for women and 68 kg for men. These values are shown in Fig. 4a (men) and 4b (women), where they are compared with the P/E ratio of four diets: UK adult omnivores; UK adult vegetarians (meat-free); the average diet of India; and the average diet of West Bengal. The P/E ratio for each diet has been adjusted for PDCAAS as necessary, for each age group, using age-dependent amino acid scoring patterns. An indication of the variability of the intake values is shown for the UK intake data ($\pm 1SD$), which has been calculated from the variance of the P/E ratio of the intakes as reported by Jackson and Margetts¹². In Fig. 5 we show how adult body weight influences the comparison within the range likely to occur in a developing country.

Estimation of risk of deficiency

Table 3 shows estimates of risk of deficiency calculated from the mean P/E ratio of the requirement ($EAR_{protein}/EAR_{energy}$), for men and women of various ages (including the age where teenage girls first become pregnant), weight (large and small) and at three levels of physical activity, for the four dietary groups: UK adult omnivores; UK adult vegetarians (meat-free); the average diet of India; and the average diet of West Bengal. The estimates of risk are approximate, as they are based on assumed normal (or at least symmetrical) distributions. The variances of the two UK diets are as reported, whereas they are estimates for the two Indian diets, assuming the same variance as the UK diets.

Groups at risk of deficiency

Inspection of Figs 4 and 5 and Tables 2 and 3 reveals the following points.

1. Within populations, on the basis that the food available and consumed does not vary markedly between children and adults of all ages, protein deficiency

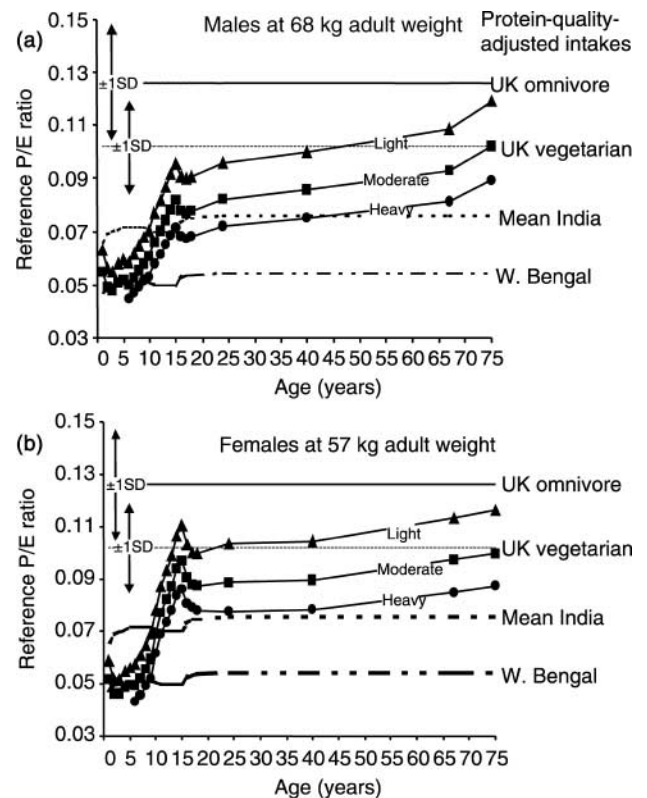


Fig. 4 Reference protein/energy (P/E) ratios for women and men compared with the adjusted P/E ratios of diets. Reference P/E ratios values calculated as in Table 2 on the basis of National Center for Health Statistics/World Health Organization median weights-for-age as reported by Torun *et al.*⁴⁴, energy requirements calculated from Schofield equations adjusted for physical activity level (PAL) values of 1.5, 1.75 and 2.0 for both males and females¹¹, and with protein requirements calculated as in Fig. 1. The adjusted P/E ratios of diets are based on the gross P/E ratios and digestibility values shown in Table 1, and biological values adjusted assuming lysine to be the limiting amino acid and with the age-related scoring pattern shown in Fig. 3. SD, standard deviation

(i.e. a P/E ratio of the diet that is less than the reference P/E ratio) is most likely in elderly sedentary women and least likely in moderately active young children (Table 2). This is the opposite of what is usually assumed. As shown in Table 1, the P/E ratio of breast milk, which can be assumed to be a close match to the desirable P/E ratio of infants, at 0.06 is half that of the adult diet. However, for infants and very young children the assumption has to be made that the diet is sufficiently energy-dense that the bulk of the diet does not limit consumption to a quantity that fails to satisfy energy requirements. After elderly sedentary women, the next most vulnerable female group is the adolescent at 15 years, an age when pregnancies may begin (Fig. 4).

2. The P/E ratio of the UK omnivore diet exceeds the reference P/E ratio for most individuals except for heavier, sedentary elderly women (70 kg) (Fig. 4), for whom the mean P/E ratio of the intake, at 0.126, is slightly less than the reference P/E ratio of 0.129. For this

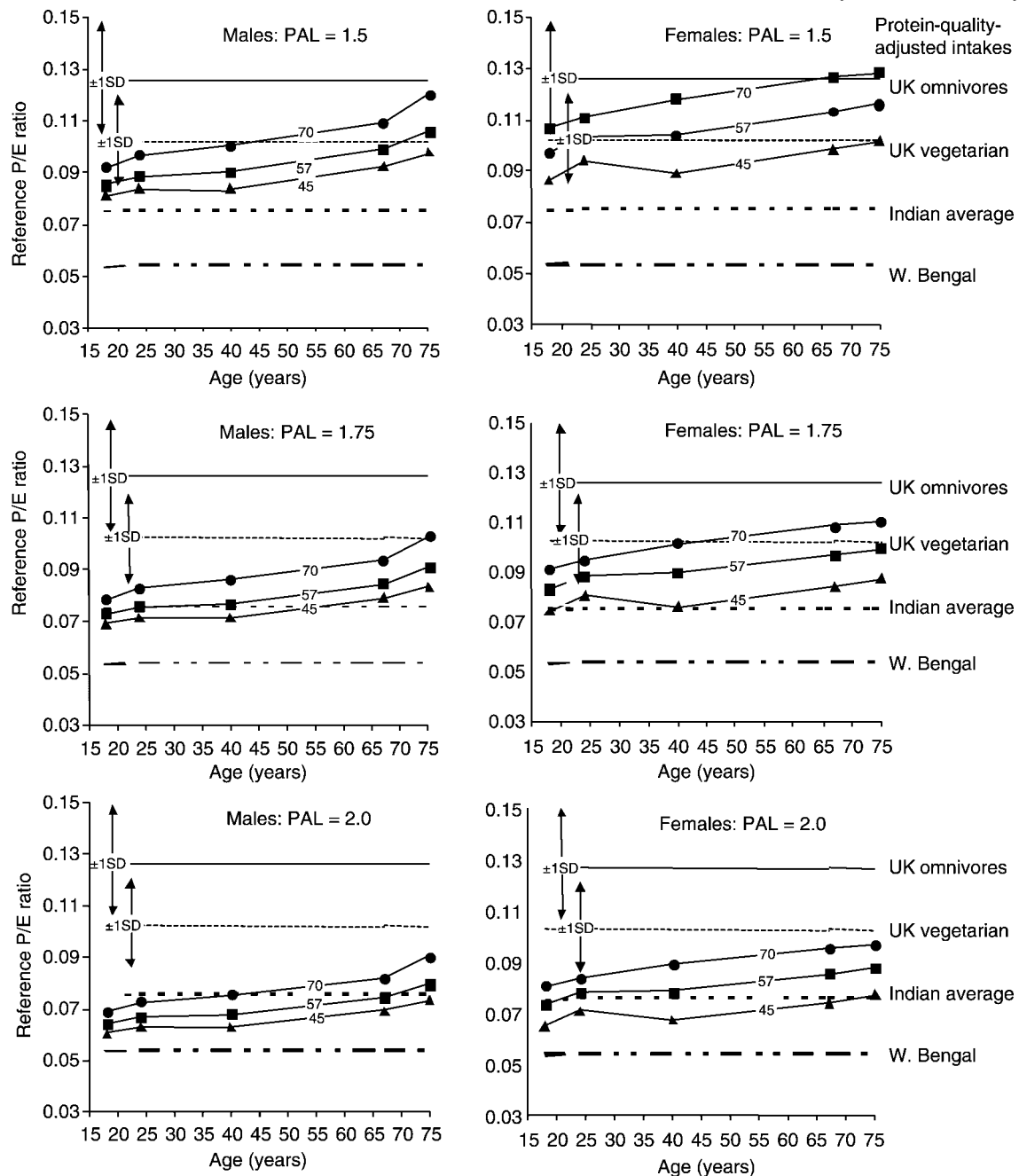


Fig. 5 Influence of adult body weight on comparisons of reference protein/energy (P/E) values with protein-quality-adjusted P/E ratios of diets. See legend to Fig. 4 for origin of values. PAL, physical activity level; SD, standard deviation

group (Table 3), the mean P/E ratio of the requirement is 0.094, and the risk of deficiency (P/E ratio of the intake below the mean ratio of the requirement $EAR_{\text{protein}}/EAR_{\text{energy}}$) is about 5%. For sedentary men weighing 70 kg and sedentary 15-year-old women, the risk of deficiency is about 3% in each case.

3. The P/E ratio of the UK vegetarian diet exceeds the reference P/E ratio for most males apart from sedentary older people with a body weight of 57 kg or higher, but is less than the reference P/E ratio of sedentary adolescent and adult females of any age at a body

weight of 57 kg or higher (Fig. 4). For a 70-kg, 75-year-old women, risk of deficiency is 31% if sedentary, 9% if moderately active and requires an intake corresponding to a PAL of 2 before risk is acceptably low (3%). The corresponding risks for men at this age and weight are 18, 5 and 1% for PAL values of 1.5, 1.75 and 2 (Table 3). For smaller, 70-year-old men (e.g. 45 kg), risk would be acceptable at any intake, but for women at this weight and age risk would be acceptable if active but would be 4% if sedentary. For large, 70-kg, adolescent females aged 15 years, risk is 19% at a PAL value of 1.5, 5% at a

Table 3 Estimates of risk of dietary protein deficiency for population groups of various ages and body weights consuming diets within the UK and India, as assessed from the protein/energy (P/E) ratio of intakes and mean P/E ratio of requirements

Dietary intakes		UK omnivore	UK vegetarian	Mean India	W. Bengal	Mean India	W. Bengal
Quality-adjusted P/E ratio		0.126	0.102	0.076	0.054	0.088¶	0.070¶
Coefficient of variation (%)*		16	16	16	16	16	16
Standard deviation		0.0202	0.0164	0.0121	0.0087	0.0121	0.0087

Requirements and risk of deficiency										
	Age (years)	Weight (kg)	PAL†	Mean P/E ratio‡	% at risk§					
Women	75	70	1.5	0.094	5	31	93	100	69	100
	75	70	1.75	0.081	1	9	65	100	27	89
	75	70	2.0	0.070	0	3	33	97	7	52
	75	45	1.5	0.074	1	4	45	99	13	69
	75	45	1.75	0.064	0	1	16	86	2	23
Men	75	45	2.0	0.056	0	0	5	57	0	5
	75	70	1.5	0.087	3	18	83	100	48	98
	75	70	1.75	0.075	1	5	47	99	14	72
	75	70	2.0	0.066	0	1	20	91	3	31
	75	45	1.5	0.071	0	3	34	97	4	33
Women	75	45	1.75	0.061	0	1	11	78	0	6
	75	45	2.0	0.053	0	0	3	45	0	1
	18	70	1.5	0.078	1	7	56	100	20	82
	18	70	1.75	0.067	0	1	23	92	4	35
	18	70	2.0	0.058	0	0	7	68	1	9
	18	45	1.5	0.063	0	1	14	84	2	21
	18	45	1.75	0.054	0	0	4	49	0	3
	18	45	2.0	0.047	0	0	1	21	0	0
	15	70	1.5	0.088	3	19	83	100	49	98
	15	70	1.8	0.075	1	5	47	99	14	72
Men	15	70	2.0	0.066	0	1	20	91	3	31
	15	45	1.5	0.071	0	3	34	97	8	54
	15	45	1.8	0.061	0	1	11	77	1	14
	15	45	2.0	0.053	0	0	3	45	0	3
	18	70	1.5	0.067	0	2	23	93	4	36
	18	70	1.75	0.057	0	0	6	64	1	7
	18	70	2.0	0.050	0	0	2	32	0	1
	18	45	1.5	0.059	0	0	8	70	0	5
	18	45	1.75	0.050	0	0	2	33	0	1
	18	45	2.0	0.044	0	0	0	12	0	0
Infants	5	18	1.5	0.042	0	0	0	7.6	0	0
	5	18	1.75	0.033	0	0	0	2.4	0	0

* Values from Jackson and Margetts¹² for UK diets, with the same value assumed for the Indian diets.

† Physical activity level (PAL) assumed in calculating the P/E ratio of the requirement.

‡ Calculated from mean protein requirements and estimated basal metabolic rate times PAL values as: $EAR_{\text{protein}}/EAR_{\text{energy}}$.

§ The fraction of the intake distribution below the mean requirement, calculated assuming a normal distribution.

¶ Calculated assuming lysine requirement of $18 \text{ mg kg}^{-1} \text{ day}^{-1}$, with 27 mg lysine per g protein.

PAL of 1.75, and 1% at a PAL of 2, but the risk is low for a small, 15-year-old female who weighs 45 kg.

- The P/E ratio of the average Indian diet exceeds the reference P/E ratio for infants and children below the age of 10 years (Fig. 4). However, for women of average weight, it is less than the reference P/E ratio for adolescents or adults at any age unless PAL values are >2 , and would require body weight to be less than 57 kg and PAL values higher than 1.75 (Fig. 5). For men of average weight (Fig. 4) the P/E ratio of the diet is less than the reference P/E ratio for adolescents or adults at any age or activity level apart from very active (PAL ≥ 1.75). Young adults, <50 years, would have to have a body weight below 57 kg and PAL values greater than 1.75 for the P/E ratio of the diet to meet the reference P/E ratio (Fig. 5). For large (70 kg), elderly men and women the risk of deficiency is 20–93%

according to the level of physical activity. For small, elderly, sedentary men and women, the risk is 34–45%, with acceptably low levels of risk at moderate or intense levels of physical activity (Table 3). Younger women at 70 kg need to be very active before risk is acceptable, since, even with a PAL of 2, the risk is 7%. For the large adolescent, risk is significant at all levels of intake, between 20 and 83%. Even smaller adolescents (45 kg) have a significant risk if sedentary, 34%, and a high level of activity has to be achieved before the risk becomes acceptable. For large (70 kg) younger men the risk is appreciable if sedentary, 23%, or with moderate activity, 6%, and for smaller men if sedentary, 8%.

- The P/E ratio of the worst diet considered here, that of West Bengal, is less than the reference P/E ratio of any age, sex or activity group of average weight apart from very active (PAL = 2) 5–7-year-olds (Fig. 4). The body

weight of 18-year-old men would need to be below 45 kg with a PAL value of <2 for there to be negligible risk, since at 45 kg and a PAL = 2 the risk is 12% (Table 3). For the older male (75 years), even with a small body weight (45 kg) and a PAL of 2, the risk is 45%. For women this diet appears grossly inadequate at any feasible level of food intake and at any body weight down to the lowest levels likely to occur in India. For the most physically active, small (45 kg) woman the risk is 45% at 15 years and 21% at 18 years. For older women, even those with a small body weight and intense physical activity, the risk is very high, 57%.

Discussion

In this paper we have explored some of the problems involved in the derivation of recommendations for dietary protein, which exemplifies some of the difficulties inherent in moving from nutrient-based to food-based dietary guidelines. Although both nutrient-based and food-based guidelines are developed logically within a coherent system, they cannot always be brought together simply. There are a number of reasons for this, some of which have been explored here. Importantly, total food consumption is directly related to energy requirements, which vary with a range of physiological and lifestyle factors. By contrast, the requirements for a nutrient are usually expressed in relation to body weight. Hence, often there is no simple linear relationship between the need for the nutrient and the need for energy, as consumed in many diets. For protein and other nutrients, the assumption is made that there is no correlation between the requirement and the intake. Making this assumption carries major implications and leads to the conclusion that, for many populations where there is no obvious evidence of protein deficiency or poor nutritional status, there is a significant risk of dietary protein deficiency as the current level of protein consumption is less than the derived value for a safe level of dietary protein. This makes it clear that current approaches cannot be correct, and require major revision.

We assessed the adequacy of dietary protein to determine the extent of risk of deficiency, using the approaches currently adopted by national groups and expert consultations of the international agencies. The likely level of protein consumption has been indexed to reference values for energy expenditure, and hence energy and food consumption. We conclude that in the context of risk assessment, we have defined a significant problem. It is clear that, based on current definitions of the metabolic requirements for protein and amino acids, none of the diets considered is adequate to meet the requirements of everyone in the population. Thus, large, older sedentary women are at risk when consuming the UK omnivore diet. Apart from those with low body weight, all sedentary adolescents and adult women are at risk

when consuming the UK vegetarian diet. Few within the Indian population could meet their requirements from their habitual diet, and none in West Bengal, regardless of their size or activity. In other words, our calculations identify a major problem of inadequate protein consumption, in terms of both quantity and quality (digestibility and lysine requirement), in developed and especially developing countries. However, our calculations are based on a number of assumptions and these assumptions require critical scrutiny to assess their validity.

The calculations of risk shown in Table 3 are approximate, being based on limited data on dietary intakes and making assumptions about the distributions and variances of these intakes. For the two UK diets, we have assumed these values to be the same for all age groups. The most recent dietary data from the UK indicate a higher P/E ratio for the diets of older people⁴³, so that further detailed analysis is required. For the UK diet the variances of the P/E ratio are as reported, but for the two Indian diets it has been assumed that the variance of the intake is similar to that in the UK. If the variance is much lower than that assumed then the risk is reduced. It may be that with more monotonous diets, such as the rice-based diets consumed in West Bengal, there is less variation in the dietary P/E ratio: i.e. a low frequency of consumption of both protein-rich foods likely to increase the P/E ratio and of high-fat foods likely to decrease the P/E ratio. However, some widely consumed sweets have a low P/E ratio, especially if consumed with sugar syrup (i.e. *jalebi*: P/E ratio ~ 0.05). Other widely consumed foods, such as *gulab jaman* (P/E ratio 0.092) and the milk-based sweet *rossogolla* (P/E ratio 0.22), have a P/E ratio higher than that of rice (AV Kurpad, personal communication, 2002). Therefore, it would be unsafe to presume that the variance in the P/E ratio is necessarily much lower. However, as indicated in Table 3, except for extremely physically active younger women, young men of higher body weight and adult men of low body weight, the mean protein-quality-adjusted P/E ratio of the diet in West Bengal (0.054) is lower than the mean P/E ratio of the requirement. Thus, for these groups, the risk of deficiency will be greater than 50% regardless of the variance in the P/E ratio of the intake.

A second potential inaccuracy relates to the predicted energy requirements. As the basis of our calculations we have used the Schofield equations to predict BMR¹¹, recognising that these may not be appropriate for the Indian population. According to Hayter and Henry's re-examination of the BMR predictive equations, values could be 10% lower than those we have used^{45,46}. This would increase the P/E ratio of the requirements, thereby increasing the risk of deficiency compared with our calculations.

On the basis that our estimations of risk are reasonable as far as our calculations are concerned, this analysis has

identified a very high risk of protein deficiency. We are left with one of two alternative possibilities to explain this unsatisfactory situation. One is to accept these conclusions and pursue risk management by considering the need to increase the availability of adequate supplies of high-quality protein to those populations at risk of deficiency. The influence of increased consumption of legumes is shown in Fig. 3. A strategy along these lines has already been suggested by Young *et al.*⁴⁷, based on their opinion that the requirements for lysine have been underestimated and hence lysine intakes are inadequate. The other possibility is to critically examine the assumptions inherent in the conceptual metabolic framework that is currently used as the basis for defining protein requirements, and therefore underlies any conclusions: to explore and refine alternative approaches to the definition of requirements.

Are our assumptions about protein quality correct? This is an issue of considerable controversy. As indicated above, the meta-analysis of nitrogen-balance studies, assumed to represent the best available estimate of the protein requirements, failed to identify any difference in utilisation between animal and plant protein sources¹⁰. On this basis no correction would need to be made for anything other than digestibility. However, the authors of the meta-analysis argue that there are differences in individual studies in which single proteins such as rice or wheat are compared with egg, but that such differences have been obscured either by complementation of different plant amino acid patterns in mixed plant-based diets, which would remove any deficiency of plant protein mixtures, or by the overall variability of the aggregated dataset. Because of this it is argued that quality-related differences between proteins do exist. Thus, scoring the BV of dietary protein on the basis of the newly derived amino acid requirement pattern is recommended within the DRI report⁹. The fact that the mean efficiency of utilisation was identified as only 0.48 in the nitrogen-balance meta-analysis was not discussed either in the

published paper or in the DRI report^{9,10}, and this is considered further below.

Here, we have used a value for lysine requirements of 30 mg kg⁻¹ day⁻¹ likely to be recommended by FAO/WHO/UNU and slightly lower than the value of 31 mg kg⁻¹ day⁻¹ recommended in the DRI report⁹. The latter value was selected by the authors of the report from values reported in the literature, which vary from 17 to 45 mg lysine kg⁻¹ day⁻¹, and are listed in Table 4. The range reflects the use of quite different methodologies and the application of different assumptions, so that rationalising them into a single value is very difficult. As a result, the choice of 30 or 31 mg lysine kg⁻¹ day⁻¹ is to some extent arbitrary. In fact, considerable controversy exists on the selection of the appropriate value for the lysine requirement. In part, the controversy derives from complex technical arguments related to the application of different methodologies in studies where stable isotopes have been used to trace metabolism. Some of these issues cannot be resolved easily. There are, however, data from a nitrogen-balance study⁴⁹ which are reliable and free from many of the criticisms levelled at the earlier nitrogen-balance studies^{50,51}. It is generally agreed that the data from these nitrogen balances are robust, although the results have to be adjusted with a correction made for miscellaneous losses of nitrogen, using the accepted value¹⁰ of 5 mg N kg⁻¹ day⁻¹. This correction was not reported in the original study, but when the correction is made the results^{26,27} indicate a requirement for lysine of about 18 mg kg⁻¹ day⁻¹. The choice of a correction of 5 mg N kg⁻¹ day⁻¹ for miscellaneous losses would be consistent with the use of the same factor in the nitrogen-balance studies, which have been used in the meta-analysis as the basis for the determination of the protein requirements¹⁰. In the DRI report the authors argued that the reanalysis of this study 'closed the gap' between the controversy surrounding the use of nitrogen balance as opposed to stable isotope studies⁹. Surprisingly, in the DRI report, the value reported for the lysine requirement from

Table 4 Published values for the adult lysine requirement

Source	Reference	Method used	Requirement (mg lysine kg ⁻¹ day ⁻¹)
FAO/WHO/UNU	2	N balance*	12
Millward	26	N balance (reanalysis†)	18.6
Rand and Young	27	N balance (reanalysis‡)	18.7
Pencharz, Ball and co-workers	32–34	Amino acid oxidation§	37–45
Kurpad, Young and co-workers	29, 30	Amino acid oxidation¶	29–31
Millward	35, 36	Amino acid oxidation	18–23

FAO/WHO/UNU – Food and Agriculture Organization/World Health Organization/United Nations University.

* The highest of various N balance studies previously reviewed by FAO/WHO⁴⁸.

† Reanalysis of data reported by Jones *et al.*⁴⁹ adjusted for 5 mg N kg⁻¹ day⁻¹ miscellaneous losses.

‡ Reanalysis of data reported by Jones *et al.*⁴⁹ adjusted for 5 mg N kg⁻¹ day⁻¹ miscellaneous losses by one-fit regression of natural logs of the balance data. The authors also derived a higher value of 33 mg lysine kg⁻¹ day⁻¹, which they prefer, using higher estimates (8 mg N kg⁻¹ day⁻¹) of the miscellaneous losses.

§ Based on [¹³C-1]phenylalanine oxidation in adults fed graded intakes of lysine.

¶ Based on [¹³C-1]leucine oxidation in Indian adults fed graded intakes of lysine.

|| Based on [¹³C-1]leucine oxidation in adults fed either wheat or milk.

this reanalysis of the original nitrogen-balance study was $30 \text{ mg lysine kg}^{-1} \text{ day}^{-1}$. This value was not that identified in the reanalysis of Rand and Young (values ranging from 17 to $36 \text{ mg lysine kg}^{-1} \text{ day}^{-1}$, derived using curve-fitting procedures, and choice of 5 or $8 \text{ mg N kg}^{-1} \text{ day}^{-1}$ for miscellaneous losses)²⁷, although $30 \text{ mg lysine kg}^{-1} \text{ day}^{-1}$ had been proposed previously by Young and was said to be 'strongly supported' by the reanalysis of the nitrogen-balance data by Rand and Young. Although it was found that the use of $8 \text{ mg kg}^{-1} \text{ day}^{-1}$ was an overestimate for miscellaneous losses in a temperate climate in the meta-analysis of the nitrogen-balance data¹⁰, Rand and Young²⁷ elected to use $8 \text{ mg kg}^{-1} \text{ day}^{-1}$ for miscellaneous losses (rather than the more appropriate value of $5 \text{ mg kg}^{-1} \text{ day}^{-1}$) when deriving the requirement value of $30 \text{ mg lysine kg}^{-1} \text{ day}^{-1}$ in their reanalysis. In fact, there are at least two independent verifications of a lower value for the lysine requirement. Thus, young men fed bread-based diets with sufficient energy for weight maintenance remained fit and achieved positive nitrogen balance over 2 months on average lysine intakes of $18 \text{ mg kg}^{-1} \text{ day}^{-1}$ in one study⁵² and 20 to $30 \text{ mg lysine kg}^{-1} \text{ day}^{-1}$ in the other⁵³, with several of the subjects fed the lower intake managing just as well as those fed the higher intake in the latter study.

If the lower estimate of lysine requirements of $18 \text{ mg lysine kg}^{-1} \text{ day}^{-1}$ had been used in our calculations it would not influence the conclusions about the UK diets, because they would not be limited in lysine if the requirement were set at $30 \text{ mg lysine kg}^{-1} \text{ day}^{-1}$. However, if the lysine requirement were $18 \text{ mg kg}^{-1} \text{ day}^{-1}$, the amino acid score of the average Indian diet and the diet of West Bengal would be increased to 100, which would in turn increase the adjusted P/E ratio of the diets to 0.088 and 0.069, respectively. This would reduce the risk of deficiency as shown in Table 3. Nevertheless, even after such a reassessment of the dietary protein quality, a substantial risk of deficiency is still apparent. Thus for the average Indian diet, risk remains substantial for sedentary and moderately active, large older men and women, and for sedentary, large younger women. With the West Bengal diet significant risk of deficiency remains for most groups.

As far as digestibility is concerned the values we have used are widely accepted, but as discussed above there are important considerations that raise concern about our current understanding of digestibility. In particular, we have insufficient understanding of the extent to which there is significant metabolism of amino acids and nitrogen in the lower gut, and how this might limit our ability to measure true digestibility. For the present it is not possible to suggest alternative values, and more work is needed in this area.

Are the assumptions we have used in the calculation of reference protein requirements and risk of inadequacy secure? This is the most important and contentious issue. In deriving the reference P/E ratio the assumption is made

that, for protein, intakes and metabolic demands are independent. This is a basic and fundamental assumption for all the traditional approaches for the determination of safe levels of dietary protein. The extent to which any form of adaptation might operate is the key, and most controversial, issue. In our view there are important adaptive metabolic responses to changes in the protein content of the diet. We have proposed mechanisms which, if correct, would lead to a measure of interdependence of intake and metabolic requirement. These include both changes in the demand as intake changes⁵⁴ and changes in the extent to which the demand is met from the diet as opposed to endogenous sources²¹. If such adaptive mechanisms do exist, the magnitude of the reference P/E ratio would be reduced.

The implications of adaptation have recently been explored in terms of an adaptive metabolic demand model for protein and amino acid requirements⁵⁵. Within this model the apparent inefficient utilisation of protein, regardless of its source, is explained in terms of incomplete adaptation to test diets used in multilevel nitrogen-balance studies. Thus measurements of protein utilisation within this adaptive metabolic demand model do indicate values for the efficiency of utilisation of milk and wheat that are more consistent with animal data, although wheat is utilised better than would be predicted^{55,36}. The implications of such a model for risk assessment, compared with the approach used conventionally, are shown in Fig. 6. Thus, within the currently accepted approach to determining protein requirements (Fig. 6a), the nitrogen-balance data are interpreted assuming no relationship between intake and requirement. The mean requirement and the between-subject variance are identified and used as the basis for determining the RNI, defined at the 97.5th percentile of the distribution. For an individual, risk of deficiency will increase as intake falls below the RNI. However, within an adaptive metabolic demand model the nitrogen balance data are interpreted differently. As indicated in Fig. 6b, it is assumed that much of the between-subject variability in short-term balance studies is due to incomplete adaptation to the sub-maintenance intakes fed in the balance studies. Thus, the true minimum requirement for achieving nitrogen balance would be towards the lower end of the reported range, indicating more realistic values for the efficiency of protein utilisation. If this were the case, for fully adapted population groups, true risk of protein deficiency will only increase when intakes fall below the upper range of the true minimum requirement.

Consideration of any possible effects of adaptation poses difficult issues in terms of risk management and the development of public health nutrition policy. At the present time, in the context of providing advice on safe diets, there is little merit in departing from the current approach, as discussed elsewhere⁵⁵. Certainly, caution should be exercised in any recommendation proposing

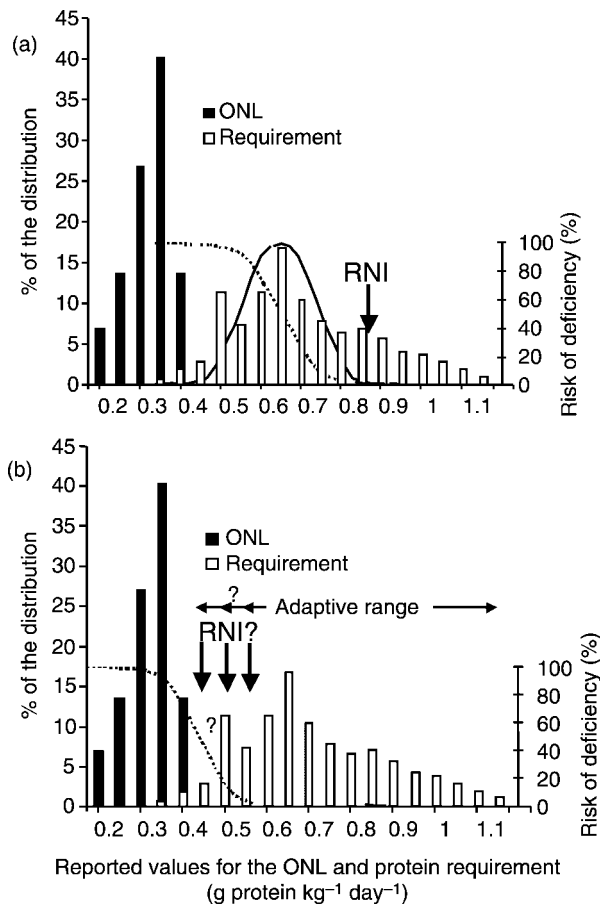


Fig. 6 Distribution of reported values for protein requirements and obligatory nitrogen loss (ONL), and calculation of the risk of deficiency for an individual for current (a) and adaptive metabolic demand model (b) of protein requirements. The bars are the distribution of reported mean values for the ONL (filled bars, 15 studies on $n=273$ subjects) and the individual values of intakes for nitrogen equilibrium expressed as protein equivalents (unfilled bars, $n=224$ individual subjects from 32 studies, after a 5% trim of outliers) from a meta-analysis of nitrogen-balance data reported by Rand *et al.*¹⁰. (a) Most of the variation in the data is judged to be methodological, with individual variation estimated to be only 12%. On this basis and with the median value estimated at $0.66 \text{ g protein kg}^{-1} \text{ day}^{-1}$, a normal distribution of requirements is shown (solid line). The Reference Nutrient Intake (RNI) is shown, defined as the 97.5th percentile of the distribution, and the risk of deficiency for an individual (broken line) is calculated assuming no correlation between protein intake and requirement. Thus risk of deficiency will increase as intakes fall below the RNI, reaching 50% at an intake equal to the Estimated Average Requirement (EAR) ($0.66 \text{ g protein kg}^{-1} \text{ day}^{-1}$). (b) Most of the variation in the data is assumed to reflect incomplete adaptation to the test diets, with the true minimum requirement at the lower end of the observed range. Thus adaptation of metabolic demands to intake removes the risk of deficiency in fully adapted individuals until intakes fall to the true minimum requirement. This value is unknown, so that no distribution of minimum requirements can be drawn, but it may approach the upper range of the obligatory metabolic demands as indicated by values for the ONL. Thus the RNI for this model would be lower, possibly in the range shown, and the broken line is a suggested estimate of likely risk of protein deficiency within this model. However, the true shape and position of the risk of deficiency curve are unknown. See Millward⁵⁵ for further discussion

that lower intakes of foods containing protein be considered safe, especially since: (1) many key micro-nutrients and minerals accompany dietary protein; and (2) there may be benefit for general health and chronic disease risk reduction from protein intakes higher than the minimum for the achievement and maintenance of nitrogen balance. However, in the context of diagnostic use of requirement values, i.e. risk assessment aimed at identifying prevalence of deficit, it is important that any analysis aspires to an acceptable balance between the numbers of false positives and false negatives. This paper clearly shows very substantial risk on the basis of the current approach, which, if correct, carries extremely serious implications. Without wanting to dismiss the possibility that there is a genuine problem, in our view the assessment of the extent of this risk is probably overestimated, given that the approach even indicates significant risk in populations that are generally considered to be well-nourished in the UK. For some population groups the risk would be reduced by the selection of lower lysine requirement values, for which there is good scientific support. The acceptance of further risk reduction implicit in an adaptive metabolic demand model raises important issues and may be problematic for some. However, we would reiterate that the adaptive model is only relevant to discussion of deficiency, in terms of being unable to maintain nitrogen balance and an appropriate lean body mass, after full adaptation to otherwise nutritionally adequate diets that satisfy the demands for energy. Whether populations in this state enjoy optimal protein-related health in terms of immune function, bone health, growth in height or any other function are separate issues, which are important and need to be addressed in their own right.

Another assumption of particular importance is that, over any extended period of time, appetite and food intake are determined by levels of energy expenditure, which influence energy consumption to maintain energy balance. However, the possibility has to be considered that when the diet is marginally limiting in protein, there is a drive for protein consumption in its own right, similar to the increased appetite observed during catch-up growth⁵⁶. If meeting the needs for protein were to drive consumption, then there are important implications that need to be considered and addressed. If, for example in older people who lead a relatively sedentary lifestyle or other population groups operating at the margin, protein consumption were consistently below requirements, then any drive to increase protein consumption would be associated with an intake of energy in excess of the metabolic demands for energy expenditure. This would predispose to positive energy balance and excess adiposity with its attendant risks.

Given the considerable importance that the underlying assumptions carry for policy formulation, there is a clear and important need for continuing research into processes

and mechanisms which enable health to be achieved on protein intakes as habitually consumed. Whilst maintenance of nitrogen balance or an appropriate lean body mass must remain the major outcome measure of protein-related health, this paper has demonstrated that assessment of dietary adequacy in these terms is unlikely to be possible without a much better understanding of adaptive mechanisms. Indeed, as argued elsewhere⁵⁵, the implication that adaptive mechanisms enable maintenance of an appropriate lean body mass to be achieved at low protein intakes is that such an endpoint can no longer be used as a surrogate of adequate protein-related health, with an urgent need for research into quantifiable alternative indicators.

Acknowledgements

The authors wish to thank John Waterlow and Philip Payne for helpful discussion, George Beaton for constructive criticism, William Rand for statistical advice and for making available his database of nitrogen-balance studies, and Anura Kurpad for helpful discussion about the Indian diet.

References

- World Health Organization (WHO). *Preparation and Use of Food-Based Dietary Guidelines*. Report of a Joint Food and Agriculture Organization/WHO Consultation. Technical Report Series No. 880. Geneva: WHO, 1998.
- Food and Agriculture Organization (FAO)/World Health Organization (WHO)/United Nations University (UNU). *Energy and Protein Requirements*. Report of a Joint FAO/WHO/UNU Expert Consultation. Technical Report Series No. 724. Geneva: WHO, 1985; 150–60.
- Millward DJ, Bowtell JL, Pacy P, Rennie MJ. Physical activity, protein metabolism and protein requirements. *Proceedings of the Nutrition Society* 1994; **53**: 223–40.
- Waterlow JC. Nutritional adaptation in man: general introduction and concepts. *American Journal of Clinical Nutrition* 1990; **51**: 259–63.
- Hegsted DM. From chick nutrition to nutrition policy. *Annual Review of Nutrition* 2000; **20**: 1–19.
- Young VR, Borgonha S. Nutritional adaptation (genetic, physiological and behavioural): implications for requirements. In: Fitzpatrick DW, Anderson JE, L'Abbé ML, eds. *From Nutritional Science to Nutrition Process for Better Global Health*. Ottawa: Canadian Federation of Biological Societies, 1998; 57–160.
- Jackson AA. Limits of adaptation to high dietary protein intakes. *European Journal of Clinical Nutrition* 1999; **53**(Suppl. 1): S44–52.
- James WPT, Schofield EC. *Human Energy Requirements*. Oxford: Oxford University Press, 1990.
- Institute of Medicine. *Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids (Macronutrients)*. Washington, DC: National Academy Press, 2002.
- Rand WM, Pellett PL, Young VR. Meta-analysis of nitrogen balance studies for estimating protein requirements in healthy adults. *American Journal of Clinical Nutrition* 2003; **77**: 109–27.
- Schofield WN, Schofield C, James WPT. Basal metabolic rate: review and prediction. *Human Nutrition: Clinical Nutrition* 1985; **39C**: 1–96.
- Jackson AA, Margetts BM. Protein intakes in the adult population of the UK. *International Journal of Food Science and Nutrition* 1993; **44**: 95–104.
- Pellett PL. World essential; amino acid supply with special attention to South-East Asia. *Food and Nutrition Bulletin* 1996; **17**: 204–34.
- Food and Agriculture Organization (FAO)/World Health Organization. *Protein Quality Evaluation in Human Diets*. FAO Food and Nutrition Paper 51. Rome: FAO, 1991.
- Rosegrant MW, Leach N, Gerpacio RV. Alternative futures for world cereal and meat consumption. *Proceedings of the Nutrition Society* 1999; **58**: 219–34.
- Graham GG, Morales E, Placko RP, Maclean WC. Nutritive value of brown and black beans for infants and small children. *American Journal of Clinical Nutrition* 1979; **32**: 2362–6.
- Graham GG, Lembcke J, Morales E. Quality-protein maize as the sole source of dietary protein and fat for rapidly growing young children. *Pediatrics* 1979; **85**: 85–91.
- Maclean WC, De Romana GL, Placko RP, Graham GG. Protein quality and digestibility of sorghum in preschool children: balance studies and plasma free amino acids. *Journal of Nutrition* 1981; **111**: 1928–6.
- MacFarlane G, Cummings JH. The colonic flora, fermentation, and large bowel digestive function. In: Phillips S, Pemberton JH, Shorter RG, eds. *The Large Intestine: Physiology, Pathophysiology and Disease*. New York: Raven Press, 1991; 51–92.
- Fuller MF, Milne A, Harris CI, Reid TMS, Keenan R. Amino acid losses in ileostomy fluid on a protein-free diet. *American Journal of Clinical Nutrition* 1994; **59**: 70–3.
- Jackson AA. Salvage of urea nitrogen in the large bowel: functional significance in metabolic control and adaptation. *Biochemical Society Transactions* 1998; **26**: 231–6.
- Metges CC. Contribution of microbial amino acids to amino acid homeostasis of the host. *Journal of Nutrition* 2000; **130**: 1857S–64S.
- Millward DJ, Forrester T, Ah-Sing E, Yeboah N, Gibson N, Badaloo A, *et al.* The transfer of ¹⁵N from urea to lysine in the human infant. *British Journal of Nutrition* 2000; **83**: 505–12.
- Millward DJ. Nutrition Discussion Forum. Urine nitrogen as an independent validatory measure of dietary intake: potential errors due to variation in magnitude and type of protein intake. *British Journal of Nutrition* 1997; **77**: 141–4.
- Bingham SA. Nutrition Discussion Forum. Urine nitrogen as an independent validatory measure of protein intake. *British Journal of Nutrition* 1997; **77**: 144–8.
- Millward DJ. The nutritional value of plant based diets in relation to human amino acid and protein requirements. *Proceedings of the Nutrition Society* 1999; **58**: 249–60.
- Rand WM, Young VR. Statistical analysis of N balance data with reference to the lysine requirement in adults. *Journal of Nutrition* 1999; **129**: 1920–6.
- Dewey KG, Beaton G, Fjeld C, Lonnerdal B, Reeds P. Protein requirements of infants and children. *European Journal of Clinical Nutrition* 1996; **50**: 5119–50.
- Kurpad AV, El-Khoury AE, Beaumier L, Srivatsa A, Kuriyan R, Raj T, *et al.* An initial assessment, using 24-h [¹³C]leucine kinetics, of the lysine requirement of healthy adult Indian subjects. *American Journal of Clinical Nutrition* 1998; **67**: 58–66.
- Kurpad AV, Raj T, El-Khoury A, Beaumier L, Kuriyan R, Srivatsa A, *et al.* Lysine requirements of healthy adult Indian subjects, measured by an indicator amino acid balance technique. *American Journal of Clinical Nutrition* 2001; **73**: 900–7.
- Kurpad AV, Regan MM, Raj T, El-Khoury A, Kuriyan R, Vaz M,

- et al.* Lysine requirement of healthy adult Indian subjects receiving long-term feeding, measured with a 24-h indicator amino acid oxidation and balance technique. *American Journal of Clinical Nutrition* 2002; **76**: 404–12.
- 32 Zello GA, Pencharz PB, Ball RO. Dietary lysine requirement of young adult males determined by oxidation of L-[1-¹³C]phenylalanine. *American Journal of Physiology* 1993; **264**: E677–85.
- 33 Duncan AM, Ball RO, Pencharz PB. Lysine requirement of adult males is not affected by decreasing dietary protein. *American Journal of Clinical Nutrition* 1996; **64**: 718–25.
- 34 Kriengsinyos W, Wykes LJ, Ball RO, Pencharz PB. Oral and intravenous tracer protocols of the indicator amino acid oxidation method provide the same estimate of the lysine requirement in healthy men. *Journal of Nutrition* 2002; **132**: 2251–7.
- 35 Millward DJ, Fereday A, Gibson NR, Pacy PJ. Human adult protein and amino acid requirements: [1-¹³C]leucine balance evaluation of the efficiency of utilization and apparent requirements for wheat protein and lysine compared with milk protein in healthy adults. *American Journal of Clinical Nutrition* 2000; **72**: 112–21.
- 36 Millward DJ, Fereday A, Gibson NR, Cox MC, Pacy PJ. Efficiency of utilization of wheat and milk protein and apparent lysine requirements determined by a single-meal [1-¹³C]leucine balance protocol. *American Journal of Clinical Nutrition* 2002; **76**: 1326–34.
- 37 Butte NF, Hopkinson JM, Wong WW, Smith EO, Ellis KJ. Body composition during the first 2 years of life: an updated reference. *Pediatric Research* 2000; **47**: 578–85.
- 38 Ellis KJ, Shypailo RJ, Abrams SA, Wong WW. The reference child and adolescent models of body composition. A contemporary comparison. *Annals of the New York Academy of Sciences* 2000; **904**: 374–82.
- 39 Platt BS, Miller DS, Payne PR. Protein values of human food. In: Brock JF, ed. *Recent Advances in Human Nutrition*. Boston, MA: Little Brown, 1961; 351–74.
- 40 Institute of Medicine. *Dietary Reference Intakes: Application in Dietary Assessment* [online], 2000. Available at <http://www.nap.edu/books/0309071836/html>.
- 41 Beaton GH. Criteria of an adequate diet. In: Shils ME, Olson JA, Shike M, eds. *Modern Nutrition in Health and Disease*, 8th ed. Philadelphia, PA: Lea & Febiger, 1994; 1491–505.
- 42 Beaton GH. Recommended dietary intakes: individuals and population. In: Shils ME, Olson JA, Shike M, Ross AC, eds. *Modern Nutrition in Health and Disease*, 9th ed. Baltimore MD: Williams & Wilkins, 1999; 1705–25.
- 43 Henderson L, Gregory J, Irving K, Swan G. *The National Diet and Nutrition Survey: Adults aged 19 to 64 years. Vol. 2. Energy, Protein, Carbohydrate, Fat and Alcohol Intake*. London: Office for National Statistics and Food Standards Agency, 2003 (<http://www.foodstandards.gov.uk/multimedia/pdfs/ndnsprintedreport.pdf>).
- 44 Torun B, Davies PSW, Livingstone MBE, Paolisso M, Sackett R, Spur GB. Energy requirements and dietary energy recommendations for children and adolescents 1 to 18 years old. *European Journal of Clinical Nutrition* 1996; **50** (Suppl. 1) S37–81.
- 45 Hayter J, Henry CJK. A re-examination of BMR predictive equations. *European Journal of Clinical Nutrition* 1994; **48**: 702–10.
- 46 Shetty PS, Henry CJ, Black AE, Prentice AM. Energy requirements of adults: an update on basal metabolic rates (BMRs) and physical activity levels (PALs). *European Journal of Clinical Nutrition* 1996; **50** (Suppl. 1) S11–23.
- 47 Young VR, Scrimshaw NS, Pellett PL. Significance of dietary protein source in human nutrition: animal and/or plant proteins? In: Waterlow JC, Armstrong DG, Fowden L, Riley R, eds. *Feeding a World Population of More than Eight Billion People: A Challenge to Science*. New York: Oxford University Press in association with Rank Prize Funds, 1998; 205–21.
- 48 Food and Agriculture Organization (FAO)/World Health Organization (WHO). *Energy and Protein Requirements*. Report of a Joint FAO/WHO Ad Hoc Expert Committee. Technical Report Series No. 522. Geneva: WHO, 1973.
- 49 Jones EM, Bauman CA, Reynolds MS. Nitrogen balances in women maintained on various levels of lysine. *Journal of Nutrition* 1956; **60**: 549–59.
- 50 Young VR. Nutritional balance studies: indicators of human requirements or adaptive mechanisms. *Journal of Nutrition* 1986; **116**: 700–3.
- 51 Millward DJ, Jackson AA, Price G, Rivers JPW. Human amino acid and protein requirements: current dilemmas and uncertainties. *Nutrition Research Reviews* 1989; **2**: 109–32.
- 52 Bolourchi S, Friedmann CM, Mickelsen O. Wheat flour as a source of protein for human subjects. *American Journal of Clinical Nutrition* 1968; **21**: 827–35.
- 53 Edwards CH, Booker LK, Rumph CH, Wright WG, Ganapathy SN. Utilization of wheat by adult man: nitrogen metabolism, plasma amino acids and lipids. *American Journal of Clinical Nutrition* 1971; **24**: 181–93.
- 54 Millward DJ. Metabolic demands for amino acids and the human dietary requirement: Millward and Rivers (1988) revisited. *Journal of Nutrition* 1998; **128** (Suppl. 12): 2563S–76S.
- 55 Millward DJ. An adaptive metabolic demand model for protein and amino acid requirements. *British Journal of Nutrition* 2003; **90**: 249–60.
- 56 Millward DJ. A protein-stat mechanism for the regulation of growth and maintenance of the lean-body mass. *Nutrition Research Reviews* 1995; **8**: 93–120.