Macronutrient Intakes as Determinants of Dietary Protein and Amino Acid Adequacy

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ABSTRACT It has long been known that dietary amino acid adequacy is markedly influenced by energy balance but in recent years the importance of this has been generally underestimated. Important practical issues include unintentional variation in energy intake and consequence energy balance that may be responsible for much of the apparent variability in protein requirements. Because variation in energy expenditure and intakes in subjects in energy balance may influence nitrogen balance (NB), a framework for evaluating studies of protein or amino acid adequacy in relation to the level of energy intake needs definition. The common assumption that the type of energy influences protein utilization is probably incorrect with fat as effective as carbohydrate in maintaining NB at energy maintenance. A more difficult conceptual issue relates to the use of protein:energy (P:E) ratios in evaluating adequacy of intakes in relation to requirements. This is necessary given that protein intakes are determined by overall food energy intake that varies markedly throughout the life cycle and with lifestyle. For any diet that might be considered limiting in protein, population groups most likely to be at risk are those with the lowest energy requirements, the sedentary elderly. Thus, increased amino acid density of diets becomes more important for this population, and increased physical activity and higher food intakes at energy balance are likely to reduce the extent of any deficiency. Modeling of the implications of proposed protein and amino acid requirement values for likely risk of deficiency by comparing P:E ratios of intakes and requirements implies high levels of deficiency risk in both developing and developed population groups. This raises the question of whether proposed values for the lysine requirement need to be revaluated and consideration given to the extent to which adaptive mechanisms might enable the metabolic requirement for protein to be met from current intakes. J. Nutr. 134: 1588S–1596S, 2004.

KEY WORDS: protein requirements amino acid requirements energy intake energy expenditure protein-energy ratios protein quality protein deficiency adaptation lysine

The importance of energy intake for the effective utilization of dietary protein was long recognized (1,2) and although some attempts were made to develop a framework incorporating both energy and protein requirements (3) in the last two decades there was minimal discussion, possibly because it involves difficult concepts. Thus, the dietary adequacy of amino acids and protein is currently assessed by comparing intakes with requirements that are expressed as a fixed function of body weight for the various population groups of interest and that, with the exception of growth, pregnancy, and lactation, are assumed to be independent of age, gender, body weight, lifestyle, and patterns of behavior. In fact on the basis of well-established evidence it is likely that this is not only overly simplistic but can also lead to inappropriate judgments about likely risk of deficiency. There are two major problems that in the past were not sufficiently addressed. These are: a) the influence of energy balance and b) the influence of energy expenditure at energy balance on amino acid and protein adequacy.

Influence of energy balance on amino acid and protein adequacy

Adults. The first problem that is conceptually straightforward but that poses considerable practical difficulties is the long-recognized influence of energy balance on NB. Thus, NB studies need to be conducted in individuals in energy balance, because if energy requirements are underestimated then protein requirements will be overestimated, and vice versa. FAO/WHO/UNU (3), drawing mainly on the work of Calloway (1,2), estimated the magnitude of the impact of energy intake on NB both below and above N-equilibrium to be 1–2 mg of retained N/kcal. More recently Pellett and Young (4) evaluated all published NB studies in adults where protein and energy intakes were varied from 2100 kcal (8.7 MJ) and 18 g protein to ~4200 kcal (17.5 MJ) and 90 g protein/d. For the single data set of 361 balances, increases in both energy intake (EI) and nitrogen intake (NI) appeared to be separately and individually
effective in improving NB. Multiple linear regression indicated that 53% of the variation in NB could be explained by NI and EI in combination: i.e.,

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NB = 0.171 NI + 1.006 EI - 69.13.
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This equation indicates that protein requirements for balance at daily energy intakes of 30, 45, and 60 kcal/kg (125, 188, and 250 kJ/kg) are 1.42, 0.87, and 0.32 g/kg/d. Also the equation indicates that the energy requirement for the median N and consequent protein requirement reported in the recent meta-analysis of NB studies in adults (5), which was 0.66 g protein·kg⁻¹·d⁻¹, (105 mg N·kg⁻¹·d⁻¹) is 51 kcal (214 kJ)·kg⁻¹, a value equivalent to a physical activity level of 2.1 for a young male at 70 kg. This suggests that the intercept may be somewhat low. The slope implies an EI-NB equivalence of 1 mgN·kg⁻¹·d⁻¹ gain per extra 1 kcal·kg⁻¹·d⁻¹ intake. Thus, for a moderately active young adult male with an estimated energy requirement of 45 kcal·kg⁻¹·d⁻¹ [i.e., 1.8*predicted basal metabolic rate (BMR)], the likely error of ±10% in estimating BMR and consequent energy needs, i.e., 4.5 kcal·kg⁻¹·d⁻¹, would account for a variability in NB of ±4.5 mgN·kg⁻¹·d⁻¹, (according to a slope of 1 mg NB per 1 kcal EI), equivalent to a variability in requirement of 10 mg·kg⁻¹·d⁻¹. Compared with the total variability reported in the meta-analysis of NB estimations of the protein requirements (5), the error in establishing energy balance is about one-third of the total between individual variance (SD = 31.9 mgN·kg⁻¹·d⁻¹), or ~85% of the estimated true between individual variance. In practical terms in multilevel NB studies aimed at measuring protein or amino acid requirements, actual rates of energy expenditure and consequent energy requirements are never measured, instead body weight monitoring is the usual measure of energy sufficiency or deficiency during the study. In this case how well energy balance maintenance was maintained would depend on the vigilance of body weight monitoring in what are usually short-term studies. An overestimate of 4.5 kcal·kg⁻¹·d⁻¹ could result in 0.5–1 g of tissue gain·kg⁻¹·d⁻¹ equivalent to 0.25–0.5 kg of weight gain per wk for a 70-kg adult. Clearly this would be substantial weight gain if maintained but might be considered within the normal range in a short-term study.

**Children.** In children given the capacity for lean tissue growth the likely influence of energy balance on protein and amino acid requirements are more complex for several reasons. Firstly protein deposition is in part endogenously regulated, mainly as a consequence of height growth (6) rather than a simple direct consequence of dietary protein and energy intake. Indeed, this can enable positive NB to occur in negative energy balance. This is well documented in growing animals (7) and weight gain. The fivefold differences in the required energy intake, there will be variable ratios between maintenance and growth components of the metabolic demand for protein and amino acids, so that the balance between dispensable and indispensable amino acid utilization will be influenced. Thus, judgments need to be made about appropriate rates of protein deposition and consequent demands for indispensable amino acids when evaluating experimental studies of amino acid adequacy. One example is the study of amino acid intake in children supplemented with zinc, which had become limiting for lean tissue growth (10).

Golden (11) derived equations for energy, protein, and the protein:energy requirements as a function of weight gain and the composition of the gain in an attempt to formalize the prediction of dietary protein-energy interactions in relation to rates of weight gain and the composition of deposited tissue during catch-up growth, from theoretical considerations of the energy cost of fat and lean tissue deposition. However, although such predictive tools are useful, users must recognize their limitations. Equations for the prediction of the energy requirements for weight gain can be constructed fairly accurately because reasonably accurate predictions can be made about the digestibility and metabolizability of the energy in the diets: e.g., Golden assumes energy digestibility to be 90%. Thus,

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\text{energy intake} = \left( 82 + (2.8 \text{lean fraction} + 9(1 - \text{lean fraction})) \times \text{weight gain (g·kg}^{-1}·\text{d}^{-1}) \right) / 0.9\text{kcal·kg}^{-1}·\text{d}^{-1}.
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The predicted weight gain fit with the observed outcome in terms of energy intakes and growth in recent studies on catch-up growth in infants (12). In contrast, for protein, the need to assume a single value for the efficiency of protein utilization (Golden assumes 70%) as opposed to the wide range of actual efficiency that can occur means that the predictive value of his equations can be quite poor especially during rapid weight gain. Unlike in healthy adults or normal children, during rapid weight gain there is ample evidence in the literature suggesting marked variability in N-utilization that is not a function of biological value or digestibility. One example is the change in growth rates with the zinc supplementation referred to above. Marked changes in N-utilization is implied in these studies. There are, in fact, complex interactions between metabolic demands for energy and protein and dietary energy and protein supply during catch-up growth that influence the composition of deposited tissue; variation in tissue composition implies variation in N-utilization. Intakes at these high growth rates are to some extent variable due to voluntary changes in intake as a result of appetite regulation. This may be particularly important as the P:E ratio increases and the dietary protein supply approaches the maximum metabolic demand for protein in terms of lean tissue growth.

What is most important from the perspective of protein and amino acid utilization in children is that because of variable weight gain and protein deposition, at least in part a function of energy intake, there will be variable ratios between maintenance and growth components of the metabolic demand for protein and amino acids, so that the balance between dispensable and indispensable amino acid utilization will be influenced. Thus, judgments need to be made about appropriate rates of protein deposition and consequent demands for indispensable amino acids when evaluating experimental studies of amino acid adequacy. One example is the study of amino acid...
Influence of energy expenditure at energy balance on amino acid and protein adequacy

The assumption made in most published studies is that the variation in energy intake involves changes in energy balance, i.e., changes from a deficit to an excess. Metabolically this means in the most simplest of terms that at the low energy intake amino acids are diverted to energy yielding reactions, whereas at the excess energy intake level amino acids may be deposited in association with the weight gain and the development of obesity. Most of the literature examined by Pellet and Young (4) falls into this category but it can be assumed that some of the variation in energy intake was at energy balance, i.e., associated with similar variation in energy expenditure through variable physical activity. This is an important issue that may confound some of the studies and that in any case is an important issue in its own right.

Firstly the effects of variable rates of energy intake at balance, i.e., with variable expenditure, may be of great practical importance in that in the developing world, rates of energy expenditure may be higher than those associated with the more sedentary lifestyles in the developed world. Thus, populations in the developing world, often at risk of nutritional adequacy are likely to have lifestyles associated with high rates of energy turnover compared with low rates of turnover in well-nourished but sedentary developed populations. The question therefore is: what is the effect of energy turnover, e.g., a subject’s physical activity level (PAL) value on NB? Although it is well known that inactivity associated with bed rest results in loss of lean body mass (LBM), data relating to the extent to which physical activity can optimize dietary protein utilization in normal adults are limited. The most quoted study is that of Butterfield and Calloway (15) who examined the influence of exercise on NB in subjects on lower than average protein intakes, by comparing the influence of an exercise program on NB at zero energy balance and at a fixed level of energy supplementation. NB was improved by the exercise in each case and the changes in LBM were by and large consistent with the NB data. However, given that the NBs were not randomized, the improving NB with increasing energy intake and expenditure may have represented continuing adaptation to the lower than usual protein intakes in the studies. This raises concern about the strength of the authors’ conclusions that physical activity improves protein utilization.

The question of identifying protein requirements associated with high levels of physical activity, in sport for example, is a complicated issue that has yet to be resolved. In our previous assessment of the literature (16) we concluded that the relationship between protein needs for overall balance and physical activity is represented by a U-shaped curve, with needs increasing in both the inactive and intensely active state although the extent of any increase with activity is reduced by increasing in both the inactive and intensely active state. Protein needs may also be increased by increasing dietary protein intake that also often accompanies high levels of physical activity.

The possible interdependence of energy and protein requirements is a problematic aspect of the impact of energy on amino acid adequacy because there is no agreed framework for evaluating studies of protein or amino acid adequacy in relation to variation in energy intakes in a subject in energy balance. Most NB studies are predicated on the view that energy and protein requirements are unrelated and can be separately defined at least in terms of minimal values. A case in point is the series of long-term NBs reported by Garza et al. (17,18). In these studies additional energy intake was needed to restore NB and lower plasma transaminases in subjects fed 0.57 g·kg\(^{-1}·d\(^{-1}\). The authors argue from conventional wisdom that the protein intake was inadequate. In fact it is equally logical to argue that energy requirements are variable according to the dietary protein level especially at low-protein intakes and that the subjects were initially fed too low an energy intake. In human studies, in which measurements of actual energy balance can only be made very approximately with imprecise measurements of body energy and compositional changes, monitoring the consequences of variable energy or NB is difficult; the extent to which increases in body weight accurately assess changes in lean body mass is also very difficult to assess. There is abundant animal evidence for positive NB at high-protein low-energy intakes in animals losing weight and in negative energy balance and vice versa (7). The lack of any agreed framework also applies to subjects at high levels of energy intakes where the subjects are not gaining weight, i.e., at energy balance. Is energy balance the appropriate criterion or balance with a range of energy expenditures, say 1.7–2.2* BMR? In the Minnesota Bread Study (19), weight, fitness, and positive NB was maintained for 50 d on a diet of which 90–95% protein, (total = 0.94 g·kg\(^{-1}·d\(^{-1}\), derived from whey. This provided only 18 mg lysine·kg\(^{-1}·d\(^{-1}\). The subjects increased their physical activity, because of the “spring weather,” requiring 3835 kcal/d (provided from nonprotein calories) for energy balance and weight maintenance. My view is that this intake was in a normal range of activity so that in the absence of weight gain this was a valid test of the adequacy of an intake of 18 mg lysine·kg\(^{-1}·d\(^{-1}\). To argue as others have (20) that “the high dietary energy intakes provided by the experimental diet confound interpretation of the N-balance data…” limits measurements of amino acid adequacy to subjects in the lower range of usual physical activity. Such judgments must be justified because they imply that we must define protein requirements in relation to physical activity levels and consequent energy intakes. The question of whether we can continue to define protein requirements independent of likely energy intakes and expenditure must be considered.

Effects of types of energy on protein utilization

The classical view, which derives from early studies by Cathcart (21) and Munro (22), is that the type of energy source is important in determining the influence of energy on N-utilization and balance at least in the submaintenance range of protein intakes. Carbohydrate is deemed to be better than fat as a result of the greater insulinogenic influence of carbohydrate (23) and because of the primary importance of insulin in mediating postprandial protein utilization. Certainly variation in the efficiency of protein utilization appears to reflect individual variation in the amino acid stimulated insulin-mediated inhibition of proteolysis (24). The classical view is usually supported by reference to a particular NB study with varying fat:carbohydrate ratios reported by Richardson et al. (25). Isoenergetic exchange of carbohydrate with fat (increasing fat calories from 33 to 48%) in diets fed at 45 kcal·kg\(^{-1}·d\(^{-1}\) with milk protein at 0.57 g·kg\(^{-1}·d\(^{-1}\) body weight reduced NB from +0.23 g N·kg\(^{-1}·d\(^{-1}\) to −0.25 g·kg\(^{-1}·d\(^{-1}\) in healthy young men.

This view that high-carbohydrate diets are more effective than high-fat diets in promoting NB was challenged by Boirie and Beauffre (26) who argue that the Richardson study was...
misinterpreted: i.e., the higher NB during the high-carbohydrate diet was true only for those subjects whose energy and possibly protein intakes were below their requirements and who were losing weight. For subjects maintaining weight there was no difference between the two diets, with a trend toward a higher protein-sparing effect of the high-fat diet. Also in a more recent duplication of this study (27) in healthy subjects receiving 75% of their maintenance energy intake, NBs were identical whether the energy was provided by diets providing a carbohydrate (CHO) to fat ratio of 2:1 or 1:1, whereas at maintenance energy intake the protein-sparing action of the high-fat diet was highest. There is no clear explanation for the different results obtained in these two similar studies. In the clinical environment there was extensive comparisons of the protein-sparing effects of glucose as opposed to lipids during total parenteral nutrition (TPN) in adults and children. Isocaloric amounts of glucose or lipids usually given as long-chain triglycerides (LCT) were compared at or above maintenance levels and most studies showed an equivalent nitrogen-sparing effect in these two substrates, both in adults and children using various fat to CHO ratios. As pointed out by Boirie and Beaufreere (26), many of these trials were conducted in patients with an inflammatory state so that the relevance to conventional feeding in healthy subjects might be questioned. However, other recent studies in noninflammatory patients or in well-nourished children under long-term TPN demonstrated that a fat-glucose regimen spares more nitrogen than glucose alone. Thus, notwithstanding the paucity of good data where N-utilization was carefully measured, most studies show that, at energy intakes at or above maintenance levels, fat and CHO exert a nitrogen-sparing effect that is at least equivalent and possibly better for fat. Only at low submaintenance energy levels is there reliable evidence that glucose could be more efficient than fat.

In fact high-fat regimes that were developed to ensure rapid weight gain in children (an energy dense high-fat diet with a fat/carbohydrate ratio up to 4:1) promotes very efficient N-utilization as do high-lipid regimes used in TPN. Also in clinical nutrition, medium-chain triglycerides were shown to be particularly effective in promoting NB. Most studies comparing fat and carbohydrate use diets with both fat and carbohydrate present although we know that some human societies successfully adapted to more or less carbohydrate-free diets (the Inuit and the Bushman). These diets pose interesting questions for protein utilization in terms of the regulation of insulin because there is no doubt that postprandial increases in insulin dominate the regulation of protein deposition.

Although it is often assumed that fat is not insulinogenic this is a misconception. Firstly, an important part of the insulin response to feeding is mediated through the enteroinsular axis by incretins, glucagon-like peptide 1 (GLP-1), and glucose-dependent insulinovertic peptide (GIP), gut hormones that may be responsible for 50% of the insulin secretion in response to feeding (28). GIP, in particular, is released in response to dietary glucose or fat with a proportionality between glucose or fat intake in a meal and secretion. Secondly, in the rat at least, insulin secretion (both glucose- and nonglucose-stimulated) appears to be fatty acid dependent in terms of both circulating nonesterified fatty acid (NEFA) concentration and NEFA species (29,30). Thus, it would appear that in the presence of some carbohydrate there are several mechanisms by which fat can stimulate insulin secretion. Because amino acids are also insulinogenic, although not to the same extent in adult humans as in the rat, we can only assume that carbohydrate-free, high-fat, high-protein diets, as traditionally consumed by the Inuit or Bushman, manage insulin secretion through dietary fat and amino acids acting with endogenous glucose. Thus, within the range of carbohydrate:fat ratios consumed as part of normal diets and within the much wider range used in the clinical environment, it appears that for now energy intakes can be considered independently from the composition of that energy as determinants of NB, thus simplifying the issue.

**Energy requirements and likely risk of protein and amino acid deficiency**

The second problem deriving from our current definition of amino acid requirements is that amino acid intakes are determined mainly by overall food energy intake that is determined by rates of energy expenditure, and these vary markedly throughout the life cycle and with lifestyle. Assuming that any evaluation of the adequacy of dietary protein presumes that energy balance has been achieved, if protein needs are expressed in terms of its nutrient density, i.e., the ratio of protein energy:total energy (P:E ratio), then evaluation of its adequacy is facilitated by comparing both dietary intake and requirements in terms of a P:E ratio. This enables those factors that influence energy requirements, i.e., age, gender, body weight, lifestyle, and patterns of behavior to be taken into account; dietary protein or amino acid adequacy can be assessed for any specific population group making the assumption that it was consumed to meet energy needs. In fact, recommendations to the public for intakes are easier to make when expressed as food-based rather than as nutrient-based guidelines.

**Expressing protein requirements as protein:energy ratios**

A protein:energy ratio approach to comparing intakes and requirements was recently evaluated in detail (31). Examples will be given here to illustrate the general principles. In Figure 1, data are shown for energy and protein requirements and, for comparison, calculated P:E ratios of requirements for females and males of average adult body weights of 57 kg and 70 kg, respectively. The changes with age are shown for energy requirements calculated from estimates of the BMR and physical activity levels of 1.5, 1.75, and 2.0 × BMR equivalent to sedentary, moderate, and heavy physical activity. The mean protein requirement is calculated assuming a constant value for maintenance (0.65 g protein/kg·d) plus increments for growth adjusted for a 61% efficiency of dietary protein utilization. From the age of 2 y, the change with age in the protein requirement is very small (15%) whereas energy requirements fall by nearly two-thirds as a consequence of the fall in BMR and can vary by 25% at any age according to the level of energy expenditure. For these reasons the calculated P:E ratio of the requirement increases markedly with age.

The P:E ratio value shown is an approximate safe level, which as discussed extensively elsewhere (31), is similar to (EAR protein + 3 SD)/EAR energy, assuming a CV of 12% for protein. In fact because BMR/kg is assumed to be lower for women than men, to fall with age in adults, and to be lower per kg in heavier than lighter adults of any age, the reference P:E ratio not only increases with age, but is higher for females than males, is higher for small compared with large adults at any age, and decreases with physical activity. In fact, the reference P:E ratio is lower in infants and children than adults, 16% lower in 50-kg compared with 70-kg adults, 14% lower in young and middle-aged men than women at the same age or weight, and 8% lower in older men than women. Thus, assuming the reference P:E ratio represents a safe or “desirable” P:E ratio that
has to be provided by the diet, a sedentary elderly woman who weighed 70 kg would require food with more than twice the protein concentration relative to energy compared with that needed by very young children. It follows 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 the needs for energy.

Calculating protein quality–adjusted protein:energy ratios of intakes

FAO/WHO/UNU is currently reexamining recommendations for protein and amino acid intakes, and revised values were recently published in a U.S. report on dietary reference intakes (DRI) (40). We recently explored 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 focusing on population groups within any country that are most likely to be at risk of protein deficiency for any estimate of the requirement (31). This approach also allows exploration of 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 particularly 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.

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) (41). Food sources with high digestibility (>95%, e.g., egg, milk, and meat) also include wheat gluten, wheat flour, and soy protein isolate whereas digestibility may be lower at 80–90% for some whole-grain cereals and legume protein isolates, and lowest at 50–80% for cereals with resistant plant cell walls, (millet and sorghum), antinutritional factors (beans), or processing and heat treatment (breakfast cereals). Overall for some plant protein sources, digestibility can reduce nutritional value, and in our general analysis of national diets we have assumed a value of 80%.

BV is a function of the amino acid score, calculated from the dietary protein amino acid pattern in relation to the amino acid pattern that is assumed to represent the metabolic demands of the body and is calculated as an age-related pattern (mg amino acid/mg protein) from age-related values for the requirements for indispensable amino acids (IAA) and for protein. Thus, the predicted BV of a dietary protein will be lower when consumed by infants than when consumed by adults because of the higher amino acid requirements of infants. For this exercise we have adopted the revised slightly higher maintenance values for protein published in the U.S. DRI report (40) and adopted a factorial approach to the protein requirements for infants and children (35). Because in practice the limiting amino acid in the human diet is likely to be lysine (the most important limiting amino acid in cereal-based diets), we have examined adjusted P:E ratios of diets are based on the reported gross P:E ratios (38,39), adjusted for assumed digestibility values and biological value, assuming lysine to be the limiting amino acid and with an age-related scoring pattern calculated on the basis of maintenance requirements of 30 mg lysine kg⁻¹ d⁻¹ and 660 mg protein kg⁻¹ d⁻¹ for lysine and protein, protein needs for growth adjusted for a 61% dietary efficiency, and a tissue lysine content of 73 mg g⁻¹ protein (35).
BV using a lysine-scoring pattern based on the increased value for maintenance of 30 mg·kg⁻¹·d⁻¹. This is similar to the value of 31 mg·kg⁻¹·d⁻¹ for the requirement for lysine identified in the DRI report (40) with a value for infants and children calculated factorially from maintenance and growth assuming the IAA composition of body protein (e.g., 73 mg lysine/g protein) (35). The overall value of dietary protein quality can then be predicted from a protein digestibility corrected amino acid score (PDCAAS) (41), a value equivalent to net protein utilization (NPU). The amount of available protein in relation to the energy content of a food protein or diet can then be predicted as a PDCAAS adjusted P:E ratio. This is in effect the value for net dietary protein calories, nDCals%, introduced many years ago (42). This provides a measure of the ability of a food to supply utilisable protein. In Figure 2 examples are shown for the PDCAAS adjusted P:E ratio of diets of UK omnivores and vegetarians, typical of the diet of nonmeat eaters in Western societies, and those typical of cereal-based diets in the developing world; i.e., the average Indian and West Bengal diets, a minimally supplemented rice-based diet. Although both UK diets are limited only by digestibility, both Indian diets are also limited by lysine that reduces the available protein energy in West Bengal to only 5.4%.

**Dietary assessment and calculation of risk of deficiency**

Assessment of risk of dietary protein deficiency within a population group is particularly difficult when dealing with nutrient density and must be conducted with care (43–45). 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: a) intakes and requirements for energy, b) intakes and requirements for protein, and c) energy and protein requirements. In fact, as discussed elsewhere (31), information that would enable this to be done within specific population groups is generally not available and assumptions have to be made. One approach is to compare the reference P:E ratio of the population of interest, as calculated in Figure 1, with the PDCAAS adjusted P:E ratio of the relevant diets. This allows consideration of this question: given existing patterns of physical activity, energy expenditure, and consequent intake, what P:E ratio is necessary to meet the protein requirements? Although there is no agreement as to the advisability of or method involved in such a calculation, the reference (i.e., safe) P:E ratio we have calculated is similar to the approach adopted in the 1985 FAO/WHO/UNU report (appendix 9A) (3) in the context of assessing individual diets. This is calculated by a formula approximating to (EARprotein + 3 SD EARprotein) / (EARenergy), where EAR is the estimated average (or median) requirement for protein or energy. If anything, this may be an underestimate of the true safe P:E ratio, and in adopting such a value we are assuming that any indications of likely protein deficiency will involve conservative judgments of dietary adequacy.

It is clear from Figure 1 that at an average adult body weight of 75 kg all adult sedentary women would be at risk of protein deficiency, with protein intakes either very similar to or below their requirements from the age of 50, when consuming a UK meat-free vegetarian diet, although intakes are well above requirements for infants and children. Moderate physical activity would increase intakes and markedly reduce risk. In contrast, the lower-protein density, poorer-quality diet of West Bengal is inadequate for most school children and adults, regardless of the level of energy expenditure and consequent food intake.

Further comparisons are shown in Figure 2. For a large elderly sedentary woman the available protein from both UK diets is below the safe requirement whereas these diets appear adequate for younger smaller women. In young men with high activity levels of moderate body size (54 kg) both UK diets and the Indian average diet appear to be adequate, but not the West Bengal diet, whereas for a moderately active 5-yr-old even the West Bengal diet may supply enough protein.

The comparisons in Figures 1 and 2 are crude and only useful in terms of allowing an initial global assessment of likely risk. An alternative semiquantitative approach makes use of the fact that the extent of deficiency for a population is approximated by the proportion of the population with an intake below the EAR, known as the EAR cut-point method (44,45). Thus, assuming that the risk percentage 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) lies on the intake distribution. For a population group with an EAR that lies at either −1 or −2 SD of the intake distribution, then there is a 16.5% or 2.5% risk of deficiency, respectively. This approach allows approximate estimates of the risk of deficiency for selected groups once assumptions are made of the distribution of the intakes for a population. In the absence of data for the developing countries we assumed normal distributions and a CV of 16%. Thus, the assessment of risk of deficiency is estimated from the mean and variance for the P:E ratio of the diet.
in relation to the mean protein requirement (EARprotein)/(EARenergy), by calculating the proportion of the population with a P:E ratio for the intake that is less than the P:E ratio for their requirement. In practice, the cumulative distribution of a value equal to the mean P:E ratio of the requirement within the distribution of P:E intakes is calculated using the NORMDIST function within Excel (Microsoft, Redmond, WA).

Figure 3 shows the principle behind this approach with examples for the UK meat-free and West Bengal diets and selected population groups. These are large and small men and women of varying ages, each at three levels of energy expenditure and consequent food energy intakes. Children 5 y of age with light and moderate activity levels are included. The assumption has been made that the two diets are normally distributed with CVs of 16%. The P:E ratios of the average protein requirements for each population group can be identified in terms of its Z-score, (number of SD below or above the mean value for the intake), and consequent risk of deficiency according to the scales shown beneath each dietary distribution. The further to the left each requirement value lies in relation to each dietary intake value, the less risk of deficiency, with an acceptably low risk when requirements are equivalent to a Z-score of -2 (2.5% risk of deficiency) or less. A more complete analysis has been published elsewhere (31) but inspection of Figures 1–3 reveal the following main points.

1. Within populations, assuming that the food available and consumed does not vary markedly between children and adults of all ages, protein deficiency (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. This is the opposite of what is usually assumed even though the P:E ratio of breast milk that 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 so that the bulk of the diet does not limit consumption to a quantity that fails to satisfy energy requirements. This is probably correct for the 5-y-old. Among children and adolescents, the most vulnerable group is the adolescent female at 15 y, an age when pregnancies may begin. Clearly smaller body size reduces risk by increasing energy intake per kg, assuming that the prediction equations for BMR accurately account for body weight variation.

2. The P:E ratio of the UK vegetarian diet is less than the safe reference P:E ratio for large older males and females with only low or moderate activity levels, implying significant risk of deficiency. The extent of this deficiency risk for a 70-kg, 75-y-old woman is 31% if sedentary and 9% if moderately active. An intake corresponding to a PAL of 2 is required before risk is acceptably low (3%). The corresponding risks for men at this age weight and activity levels are 18%, 5%, and 1%. For smaller (e.g., 45 kg) 75-y-old men, 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 a large 70-kg adolescent 15-y-old female, risk is 19% if sedentary, 5% if moderately active, and falling to 1% at a PAL of 2. For a small (45 kg) adolescent female, risk is low at any level of activity.

3. The P:E ratio of the West Bengal diet 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-y-olds. The body weight of young men would need to be below 45 kg with a PAL value of <2 for there to be negligible risk: i.e., at 45 kg and at a PAL of 2 the risk is 12%. For older men (75 y), 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 y and 21% at 18 y. For older women, even those with a small body weight and intense physical activity, the risk is very high at 57%.

**Implications of the risk assessment**

The two diets examined here represent examples from a more extensive analysis discussed elsewhere (31) that also critically evaluates the shortcomings of this approach. They represent on the one hand a diet typical of relatively affluent nonmeat-eating Western populations conforming closely to currently accepted guidelines for healthy eating and associated with low rates of chronic disease risk (38), and on the other hand a diet that is relatively poor within the range of diets within India and developing countries in general and that is consumed by a population of relatively low socioeconomic status with relatively higher rates of morbidity and mortality. However, given the poor supply of adequate housing and clean water it would be unwise to assume that such higher rates of morbidity and mortality in West Bengal are a simple function of the poor diet. What is clear and surprising in this analysis, however, is that notwithstanding the assumptions we have made in assessing the adequacy of dietary protein intake and...
risk of deficiency, the approach adopted here appears to have defined a significant problem in relation to both diets. Thus, based on current definitions of requirements for protein and amino acids, neither diet is adequate to meet the requirements of everyone in the population. Apart from those with low body weight, all sedentary adolescents and adult women are at risk when consuming the UK vegetarian diet and no one in West Bengal could meet their requirements from their habitual diet, regardless of their size or activity. This identifies a major problem of inadequate protein consumption, in terms of both quantity and quality (digestibility and lysine requirement), in developed and especially developing countries. In fact, as shown elsewhere (31), large older sedentary women are at risk when consuming the UK omnivore diet.

There are two alternative interpretations of these calculations. One is to accept the 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 as already suggested (47), based on an opinion that the requirements for lysine were underestimated in the past and that a higher value (30 mg kg\(^{-1}\)d\(^{-1}\)) is more appropriate, indicating that lysine intakes are inadequate. The other possibility is that the assumptions made and consequent values adopted in current definitions of protein and amino acid requirements are incorrect.

In fact, there is considerable controversy about the values for lysine requirements and the metabolic framework within which protein requirements are defined (14,31,48). Suffice it to say here that valid reasons can be presented for accepting a considerably lower value for the lysine requirement of \(\sim 18\) compared with 30 mg kg\(^{-1}\)d\(^{-1}\), and for defining protein requirements within an adaptive metabolic-demands framework. If the lower estimate of lysine requirements were used in our calculations it would not influence the conclusions about the UK diets, because they are not limited in lysine even with a requirement set at 30 mg lysine kg\(^{-1}\)d\(^{-1}\). However, the BV of the diet of West Bengal would be increased to 100, which would increase the adjusted P:E ratio of the diet to 0.07. This would reduce the risk of deficiency for some groups but significant risk would remain for most groups.

The most important and contentious issue relates to the issue of adaptation and consequent calculation of risk of inadequacy. Mechanisms of adaptation have been proposed (48), which if correct, would lead to a measure of interdependence of intake and metabolic requirement at intakes above a quite low level that will reduce the magnitude of the reference P:E ratio. In fact I have proposed (48) that the true minimum requirement for achieving NB is toward the lower end of the reported published range, for an adult at \(\sim 0.5\) g kg\(^{-1}\)d\(^{-1}\) or less, and that for fully adapted population groups, true risk of protein deficiency will only increase when intakes fall below the upper range of this true minimum requirement.

In the context of risk management and the development of public health nutrition policy, it can be argued that, in the context of providing advice on safe diets, there is little merit in departing from the current approach (48) with caution required before any recommendation that proposes that lower intakes of foods containing protein be considered safe, especially because: a) many key micronutrients and minerals accompany dietary protein and b) 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 NB. However, in the context of risk assessment aimed at identifying prevalence of deficit, any analysis should aspire to an acceptable balance between under- or overestimation. Without wanting to dismiss the possibility that there is a genuine problem, we proposed that the extent of this risk is likely to be overestimated, especially the significant risk in a population generally considered to be well nourished in the UK. Risk would be reduced for populations in developing countries 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-demands model raises important but controversial issues, but the adaptive model is only relevant to discussion of deficiency in terms of being unable to maintain NB 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.

Given the considerable importance that the underlying assumptions discussed here carry for policy formulation, there is a clear and important need for continuing research into processes and mechanisms that enable health to be achieved on protein intakes as habitually consumed. Indeed, whatever the true minimum protein requirement, it may be 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 growth (6). If meeting the needs for protein were to drive consumption, then in older people who lead a relatively sedentary lifestyle, or other population groups operating at the margin in which protein consumption were consistently below requirements, 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.

Conclusion

It is clear that the adequacy of protein and amino acid intakes cannot be discussed separately from the adequacy and extent of energy intake. Although the type of energy does not seem to be important, in the context of determination of requirements there is a clear need to establish a much more vigilant approach to assessment of the extent of energy balance and to an understanding of the implications of changes in energy balance during experimental procedures aimed at evaluation of protein requirements. Indeed little attention has been given to the likely influence of energy intakes in acute postprandial studies. As to the issue of variation in energy expenditure at energy balance on protein and amino acid requirements and dietary adequacy, our understanding is very limited and further research is urgently needed. In the context of an analysis of adequacy of protein and amino acid intakes consideration of overall food energy intakes is essential to any analysis of risk of deficiency so that expression of both intakes and requirements as P:E ratios affords insight into the vulnerability of specific population groups that would otherwise not be apparent and that is often counterintuitive. Thus, population groups most likely to be at risk are not infants and children but those with the lowest energy requirements, the sedentary elderly. The nutritional implications of this are that increased amino acid density of diets becomes more important for aging sedentary populations; the public health implications are that increased physical activity and higher food intakes at energy balance are likely to reduce the extent of amino acid deficiency.