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Meat or wheat for the next millennium? Plenary Lecture

The nutritional value of plant-based diets in relation to human amino acid and protein requirements

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The adequacy of plant-based diets in developed and developing countries as sources of protein and amino acids for human subjects of all ages is examined. Protein quantity is shown not to be an issue. Digestibility is identified as a problem for some cereals (millet (*Panicum miliaceum*) and sorghum (*Sorghum* sp.)) and generally is poorly understood. Direct measurements of biological value in children are reviewed and scoring is considered. Various existing requirement values for amino acids and especially lysine are reviewed, and it is concluded that stable-isotope studies do not yet provide adequate alternative values to N balance data, which for lysine are robust after recalculation and adjustment. A new maintenance requirement pattern is developed, with higher values than those of Food and Agriculture Organization/World Health Organization/United Nations University (1985) but lower values than the Massachusetts Institute of Technology pattern (Young *et al.* 1989). Calculations of age-related amino acid requirements are based on most recent estimates of human growth and maintenance protein requirements, a tissue amino acid pattern and the new maintenance amino acid pattern. These values appear valid when used to score plant proteins, since they indicate values similar to or less than the biological value measured directly in young children. When used to score plant-based diets in India, no marked deficiencies are identified. All regions score > 1 for adults, whilst for children scores range from > 1, (Tamil Nadu) from 6 months of age to 0.78 (West Bengal), rising to 0.9 in the 2–5 year old, consistent with reports that high-lysine maize supports similar weight and height growth to that of casein. Inadequate amino acid supply is not an issue with most cereal-based diets.

Lysine: Protein scoring: Plant-based diets: Children

The present paper reviews current knowledge relating to protein quality in the context of the meat or wheat debate. The question posed is: are the meat-free or low-meat plant-based diets consumed currently by minorities of vegetarians in the developed world and the majority of populations of the developing world adequate as sources of protein and amino acids for the various age-groups consuming them? The answer to this simple question is in fact difficult to formulate and subject to controversy due to uncertainty about amino acid requirements. The problem was recognized by Food and Agriculture Organization/World Health

Organization (FAO/WHO; 1991) in their review of the assessment of protein quality. They recommended that the scoring pattern to be utilized for older children and for adults could be based on values previously adopted for the preschool child, but this recommendation was only made in the absence of agreement on any other suitable values, and was presented as a strict interim measure until a better set of values could be identified.

The main reason for this problem is, in my view, because of the unsatisfactory nature of our understanding of N, protein and amino acid homeostasis. In this paper I intend to

Abbreviations: BV, biological value; FAO/WHO, Food and Agriculture Organization/World Health Organization; IAA, indispensable amino acids; MD, metabolic demand; P : E, protein : energy; RNI, reference nutrient intake.

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identify the extent and nature of the issues, and propose a way forward which, whilst not representing a complete solution, is at least an improvement on the current *status quo*.

Plant protein sources

Plant protein sources provide 65% of the world supply of edible protein (Young & Pellet, 1994), with cereal grains (47%) and pulses, nuts and oil seeds (8%) as the other major sources. Plant protein sources in the developed countries constitute a lower proportion of intake compared with animal protein (see Table 1), but are the major source in the developing countries, with cereals predominant. Of the cereals, wheat (43%), rice (39%) and maize (12%) are the main contributors (see Rosegrant, 1999).

Plant protein sources can differ from animal sources in terms of digestibility, amino acid composition, the presence of anti-nutritional factors which adversely influence digestibility and safety, and the presence of phytoprotectant factors (such as phyto-oestrogens, see Cassidy, 1996) which may be advantageous in mediating disease protection. This latter factor, together with the perceived need to limit animal fat and hence meat intakes, has resulted in a proposed increase in consumption of plant food sources as part of the healthy diet (Health Education Authority, 1996). Although it is frequently pointed out that plants can provide all human protein needs, it is nevertheless the case that the misconception persists that they are nutritionally inferior to

animal proteins. This is the result of both complex social and cultural attitudes towards meat and the scientific tradition of protein-quality evaluation in animals. In fact, the important nutritional question is not whether plant proteins can completely provide for human amino acid needs, because this has been established for all ages; rather, the question is whether this is an easy task in practice, i.e. achievable with relatively unsupplemented low-cost cereal or other staple diets available to poor developing communities, or only possible with the much-higher-cost carefully-selected mixed diets consumed by affluent vegetarians.

Protein quantity

Animal food sources are generally high in protein, so that there is a clear relationship between protein intake and the proportion of animal foods, especially meat, in the diet. As shown in Table 1, the overall protein : energy (P : E) value of the national food supply falls from 12.1% energy in the USA to 8% in a Sub-Saharan African country like Sierra Leone as the animal protein intake falls from 74 g/d to 6.9 g/d. In the UK the P : E value falls from 14.2% energy in omnivores to 12.7% in the small number of vegetarians (non-meat eaters) identified in the UK food intake survey (Jackson & Margetts, 1993). Thus, protein intakes of vegetarians are likely to be closer to the reference nutrient intake (RNI; Department of Health, 1991) and approximately 20–30% of this small sample were below the RNI.

Table 1. Protein and lysine content of diets in relation to estimates of the requirements

Country	Protein (g/d)	Protein : energy (% energy)	Protein sources						Lysine (mg/d))
			Animal		Cereal		Pulses and soyabean (g/d)	Rest (g/d)	
			g/d	%	g/d	%			
Food balance sheets*									
US	113	12.1	73.5	65.0	24.6	21.8	2.0	12.9	7598
UK	91	11.0	52.3	57.5	22.9	25.2	2.3	13.5	5815
Tunisia	91	10.9	19.0	20.9	55.6	61.2	6.0	10.3	3911
Egypt	87	9.4	12.9	14.8	59.6	68.3	5.8	9.0	3502
Brazil	66	9.3	27.1	41.1	22.6	34.3	10.4	5.8	3918
Nepal	50	10.2	7.3	14.6	34.8	69.5	3.4	4.6	2132
Bangladesh	43	8.4	4.8	11.3	33.4	78.6	2.6	1.7	1883
Sierra Leone	34	8.0	6.9	20.3	17.0	50.0	4.6	5.5	1741
Food intake data†									
UK:									
Omnivores	74	14.2	44.1	59.4	17.3	23.3	7.0	5.9	4824
Vegetarians	54	12.7	18.1	33.6	18.7	34.8	9.4	7.6	2871
India (mean)	62	11.1	3.4	5.5	47.8	76.7	7.3	3.8	2413
Tamil Nadhu	46	9.7	2.4	5.3	29.0	63.6	6.6	7.6	2006
West Bengal	53	8.8	0.8	1.5	48.0	89.9	3.2	1.4	1869
Lysine requirements (at 65 kg body wt)									
FAO/WHO (1973)									780
FAO/WHO (1991)									3770
Toronto break point (Zello <i>et al.</i> 1993; Duncan <i>et al.</i> 1996): Mean value									2795
Safe allowance									4114
MIT scoring pattern (Young <i>et al.</i> 1989)									1950
Jones <i>et al.</i> (1956) recalculated									1209

FAO/WHO, Food and Agriculture Organization/World Health Organization; MIT, Massachusetts Institute of Technology.

* FAO food balance sheets 1961–1992, as reported by Pellett (1996).

† UK data recalculated from Jackson & Margetts (1993) with lysine concentrations from Pellett (1996). Indian data from Indian National Food Intake Survey as reported by Pellett (1996).

Whether this is a problem is to some extent debatable, but it is probably unlikely, especially in developed countries, given the overall lower morbidity and mortality of vegetarians compared with meat eaters. First, with the adult protein requirement equivalent to P:E values of about 9 and 7% energy for the RNI and estimated average requirement respectively (i.e. 0.75 and 0.6 g protein/kg body weight respectively in adults (Department of Health, 1991) with an energy requirement of $1.6 \times \text{BMR}$), the lowest-protein diets shown in Table 1 fall between the estimated average requirement and RNI for adults. Second, in Table 1 all values for protein in terms of food supplies or intake indicate P:E values higher than breast-milk at 7% energy since, as shown in Table 2, only a few plant staples have a P:E value lower than 7% energy. Indeed, wheat and maize are 'high-protein foods' compared with breast-milk if the energy density issue is ignored. Furthermore, on the basis of a metabolic model for the protein requirement, which includes a substantial adaptive component varying with intake, there is by definition a correlation between intake and requirement, so that low intakes are unlikely to become associated with substantial prevalence rates of inadequacies until they fall close to the lower RNI. Most importantly, at least as far as food supply data are concerned, wheat-based food supplies, such as those in Tunisia and Egypt which have only 15–20% animal protein sources, clearly supply protein at levels close to those of predominantly animal-based food supplies, such as those for the UK. Thus, cereal-based diets, especially those based on wheat, can supply protein at levels well above the human protein requirement. As for infants and children, the wide and successful use of soyabean-based infant formulas is evidence that plant-based diets can be adequate for infants. On the other hand, the monotonous diets based on very-low-protein starchy root crops such as cassava (*Manihot* sp.) may well supply inadequate protein intakes to ensure adequate height growth (see Waterlow, 1992). In this context it is interesting to note that in such cases (e.g. the stunted Bundi orphanage children described by Malcom (1970) who were fed almost exclusively on the low-protein starchy root taro (*Colocasia esculenta*)), stunting was the only observable symptom of any nutritional inadequacy. They were otherwise healthy, without overt symptoms of kwashiorkor, in support of Golden's (1997) arguments that kwashiorkor is not a protein-deficiency disease. The issue of whether stunting in children reflects inherent inadequacies of plant-based diets as protein sources, as opposed to other nutrient inadequacies, is outside the scope of the present paper. However, as discussed later, it has been demonstrated that young children fed on one of the hybrid varieties of maize (opaque-2, sugary-2 hybrid) as their only source of protein and energy (but with mineral and vitamin supplementation) grow in height and weight at rates similar to those achieved with casein (Graham *et al.* 1990). For these reasons, and with the nutritional adequacy question limited to consideration of protein needs, then we can reasonably safely conclude that, with the exception of some starchy roots, plant-based diets available in most parts of the world are capable of providing adequate protein for all ages. Thus, protein quantity is unlikely to be an issue, and the main question of the nutritional adequacy

of plants as protein sources is limited to their quality, i.e. digestibility and biological value (BV).

Digestibility

The FAO/WHO (1991) report on protein-quality evaluation considered the methodology for measurement of digestibility, and concluded that values for true faecal digestibility obtained in rat trials correspond closely with human measurements. As reviewed recently (Millward, 1998a), the latter report showed that food sources with high (>95%) true faecal digestibility, e.g. a typical US mixed diet (egg, milk and meat), also includes wheat gluten, wheat flour and soyabean-protein isolate. Thus, once plant-cell-wall constituents are removed, the inherent digestibility of plant proteins may be indistinguishable from that of animal proteins. Lower digestibility, i.e. 80–90% (whole-grain cereals, peas (*Pisum sativum*), polished rice, soyabean flour, chickpea (*Cicer arietinum*), and pea-protein isolates) or 50–80% (whole millet (*Panicum miliaceum*), beans, breakfast cereals and developing-country mixed diets), reflect particularly-tough plant cell walls (millet and sorghum), anti-nutritional factors (beans) or processing and heat treatment (breakfast cereals).

Graham and colleagues (Graham *et al.* 1979; Maclean *et al.* 1981) working in Lima, Peru have systematically explored protein quality in young children recovering from malnutrition, and reported the digestibility of diets based on individual sources of plants relative to casein (%) as: wheat 100, maize 90, potato 82, rice 82, beans 81, sorghum 57. For beans (*Phaseolus vulgaris*), anti-nutritional factors resulted in poor digestibilities of energy as well as N, with very low overall utilization (30% of the values for casein). With sorghum the poor digestibility can be improved by fermentation and processing to some extent. Clearly, for some plant protein sources digestibility can limit nutritional value.

One issue which was arguably not sufficiently considered by FAO/WHO (1991) is that of ileal digestibility, i.e. the fact that more N enters the colon than leaves via faecal N, with the potential for loss of indispensable amino acids (IAA) through their conversion by bacterial metabolism to NH_3 and other N sources, some of which are absorbed. This process means that true digestibility of some amino acids is overestimated, with less of the dietary IAA content absorbed than is assumed on the basis of faecal N. The extent to which this loss represents an important problem is difficult to judge. MacFarlane & Cummings (1991) report that in adults on a mixed diet about 4 g N/d is absorbed from the colon, but on the basis of measurements of urea salvage in adults on mixed diets this could all be accounted for by N derived from endogenous urea hydrolysis. Endogenous ileal IAA loss (i.e. that measured in adults on a protein-free diet) is about 22 mg/kg body weight per d (Fuller *et al.* 1994), much lower than that of a young pig, and equivalent to about 5% of the IAA intake of subjects consuming the average UK intake. However, for plant protein sources which are poorly digested in the upper intestine and which increase ileal N flow to be fermented in the colon, loss of IAA may be marked.

In addition, Bingham and colleagues (see Bingham, 1997) argue that the conventional view of digestibility does not take into account variation in faecal biomass as a function of fermentable energy supply to the colon. They argue that in normal mixed diets total faecal N excretion does not increase in response to increases in dietary protein intake, and that the major determinant of faecal N output is biomass arising from fermentation in the colon by bacteria, in turn dependent on dietary NSP and other fermentable carbohydrate intake. If this somewhat surprising view of digestibility is true, then measures of digestibility in standard rat assays may not be appropriate.

This issue is further complicated by the possibility that the N which is derived from bacterial N metabolism absorbed from the colon can include IAA. Jackson and colleagues (for example, see Meakins & Jackson, 1996) have consistently shown that a major part of urea salvaged in the lower gut and returned to the systemic circulation is not recycled into further urea synthesis but retained, suggesting that the salvaged N includes amino acids. Furthermore, we have shown that in malnourished infants recovering from protein-energy malnutrition ^{15}N can be transferred from urea to the circulating lysine pool in nutritionally-significant amounts ($> 20 \text{ mg/kg}$; Gibson *et al.* 1997). Thus, the potential exists for colonic N metabolism to both remove IAA from or add IAA to the dietary supply. This makes conventional calculations of digestibility difficult to interpret. In other words, whilst the poor digestibility of staples like millet and sorghum is clear and measurable as poor faecal digestibility, for other plant-based diets with better faecal digestibility, whether actual digestibility of individual amino acids such as threonine is in fact much worse due to poor ileal digestibility remains to be established. Since ileal digestibility is difficult to measure it is usually ignored, but it may well be a serious issue and could be part of the explanation for a low BV, when it is observed.

Amino acid composition and biological value

Amino acid composition is the main determinant of the B of protein (retained N/absorbed N), a function of the balance of absorbed amino acids in relation to metabolic demands (MD). Table 2 lists the main nutritionally-significant differences between the amino acid compositions of plant protein and animal protein, shown as beef (essentially the same as human skeletal muscle), whole-body values and breast-milk. The analytical difficulty in measuring tryptophan results in some uncertainty as to whether the much higher levels of tryptophan in breast-milk compared with muscle and other food proteins is correct. Comparing the most important plant staple food sources with human tissues (assuming arbitrarily that a value for plant protein of $< 80\%$ of that for human tissue would define a clearly lower level), it appears that for threonine, the S amino acids and tryptophan only cassava and maize contain lower levels of any of these amino acids (threonine for cassava and tryptophan for maize), although at 83% the S amino acid level of soyabean is close to the 80% cut-off level. It is also clear that some plant sources contain much higher levels of these amino acids, typified by the higher S amino acids levels in cereals. However, with the exception of soyabean at 92% and potatoes at 77%, lysine levels are uniformly lower, i.e. from 36% at the lowest (wheat) to 61% for the opaque-2, sugary-2 hybrid of maize. Thus, with the exception of soyabean (S amino acid) and the common and opaque-2 maize hybrids (tryptophan), the main difference between the amino acid contents of plant sources and human tissue protein is in the lysine content. Furthermore, because cereals or starchy roots are the major world food protein sources, and the basis of all vegetarian diets, then the lysine content of diets becomes the major difference between amino acid intakes of meat-eating developed countries and cereal-eating developing countries, and of vegetarians and omnivores. The question posed by this situation is how significant are these differences?

Table 2. Protein (% energy) and amino acid contents of human tissues, breast-milk and animal and plant food sources (Amino acid contents for beef, human tissues and breast-milk are given as mg/g protein, and those for plant food sources as a percentage of the value found in human tissues)

	P:E (% energy)	Lysine	Threonine	S amino acids	Tryptophan	Biological value	Score§	Limiting amino acid
Beef	66.0	91	47	40	13			
Human tissue	—	71	41	30	13			
Breast-milk	7.0	64	50	36	22			
Soyabean	38.8	92	93	83	102	0.94†	0.86	S amino acids
Wheat	16.6	36	71	149	94	0.53‡	0.50	Lysine
Maize	13.5	41	88	97	40	0.41†, 0.79‡	0.52	Tryptophan
Mz:o2	14.0	56	107	160	55	1.05†, 0.91‡	0.81	Tryptophan
Mz:o2s2	14.0	61	—	—	71	0.96‡	0.87	Lysine
Sorghum*	10	31	92	97	92	0.65‡	0.44	Lysine
Potatoes	9.7	77	94	96	113	1.10‡	1.1	Lysine
Rice	7.2	51	90	133	86	0.86†, 0.84‡	0.78	Lysine
Yam*	6.1	58	82	92	102	—	0.82	Lysine
Cassava*	3.4	46	50	96	109	—	0.65	Lysine

mz:o2, mz:o2s2, opaque-2 and opaque-2, sugary-2 hybrids of maize respectively; P:E, protein:energy.

* Sorghum, *Sorghum* sp.; yam, *Dioscorea* sp.; Cassava, *Manihot* sp.

† Calculated relative to breast-milk from N-balance data reported by Bressani *et al.* (1973), measured in young children recovering or recovered from malnutrition.

‡ Calculated from similar data from Maclean *et al.* (1981).

§ Scoring pattern of 3–6-month-old child (See Table 3).

Direct measurement of biological value

Traditionally, rat growth assays have been used to answer this question of assessing protein quality. Since the rat growth requirement pattern (Benevenga *et al.* 1994) is, with the exception of the S amino acids, similar to that of rat mixed body proteins (Davis *et al.* 1994), rat growth trials effectively compare the composition of food proteins with that of tissue protein, because the amino acid pattern for growth must provide at least the IAA content of the tissue growth plus any extra to provide for any inefficiency of utilization or any non-growth MD. For the most part, rat growth trials have identified limiting IAA which are consistent with the differences between the amino acid compositions of plant and animal proteins. However, the relevance of rat growth trials in human nutrition can be questioned both in terms of the quantitative importance of tissue growth to overall human needs, and from the perspective of whether amino acid requirements for growth differ from those for maintenance.

In fact, as shown in Table 3, while tissue growth represents the major part of the protein requirement in the first few months of life, maintenance becomes the major part after 6 months. Thus, as might be expected in rapidly-growing young children, such as those recovering from

malnutrition, differences in dietary protein quality of various unsupplemented plant protein sources are observed. From N-balance data reported from such studies (for example, see Bressani *et al.* 1973; Maclean *et al.* 1981), the BV can be calculated relative to breast-milk. Such values are shown in Table 2. Clearly, wheat, sorghum and maize have a low BV, especially in the faster-growing children in the Maclean *et al.* (1981) studies. However, it is clear that the relatively small increases in lysine from wheat to rice have a marked impact on the BV, whilst for potato and the best maize hybrid the BV is comparable with that of breast-milk. This is the case even though lysine levels are still lower than those in human tissues, and such foods would have had a low BV in rat growth trials. Since in the children the maintenance requirement is a much larger proportion of requirement when compared with that of the growing rat, the higher BV compared with rat studies could reflect a difference in the amino acid requirement for maintenance compared with growth.

As for adults, as reviewed elsewhere (Millward *et al.* 1989), although the lower BV of wheat gluten compared with beef is observed in short-term N-balance studies (Young *et al.* 1975), differences in the BV between plant and animal proteins are much less apparent than those found

Table 3. Revised age-related amino acid requirement and scoring patterns

			Ages (months)					
			0-1	1-3	3-6	6-12	12-24	24-60
Protein requirements (g/kg body wt per d)*								
Growth			1.43	0.81	0.43	0.26	0.16	0.10
Maintenance			0.56	0.56	0.56	0.56	0.56	0.60
Amino acid requirements								
	Maintenance (mg/kg body wt)	Growth†‡ (mg/g protein)	Requirements (maintenance + growth) (mg/kg body wt per d)					
Isoleucine	18.1†	35.0	68	47	33	27	24	22
Leucine	26.3†	75.0	133	87	58	46	38	34
Lysine	18.6‡	71.0	120	76	49	37	30	26
Total SAA	16.0§	30.0	59	40	29	24	21	19
Total AAA	19.7	70.0	120	77	50	38	31	27
Threonine	15.7†	41.0	74	49	33	26	22	20
Tryptophan	3.7†	12.8	22	14	9	7	6	5
Valine	13.5†	47.0	81	52	34	26	21	18
Amino acid scoring patterns			(mg/g protein)					
Isoleucine	32.2	35.0	34	34	33	33	33	30
Leucine	47.0	75.0	67	64	59	56	53	51
Lysine	33.2	71.0	60	56	50	45	41	39
Total SAA	28.6	30.0	30	29	29	29	29	27
Total AAA	35.2	70.0	60	56	50	46	43	41
Threonine	28.0	41.0	37	36	34	32	31	30
Tryptophan	6.6	12.8	11	10	9	9	8	8
Valine	24.1	47.0	41	38	34	31	29	28

SAA, S amino acids; AAA, aromatic amino acids.

* From Dewey *et al.* (1996).

† Values computed from regression equations reported by Hegsted (1963) with addition of 0.3 g N losses assuming 60 kg body weight.

‡ Value from Fig. 2; zero balance intake for regression of all balances on log_e lysine intakes.

§ Obligatory oxidative loss (Millward & Rivers, 1988) adjusted for utilization as described by Young *et al.* (1989).

|| Tolbert & Watts (1963); 1184 mg/d expressed per kg body weight for mean body weight of 60 kg.

¶ Modified from Table 11 of Dewey *et al.* (1996) with cysteine assumed to be 50 % concentration of methionine, and tryptophan assumed to be the same as that in beef.

in children, and they are not observed in longer-term studies. Furthermore, the marked inter-individual variability precludes quantitative studies, and has certainly prevented a simple answer being found to the significance of the low lysine levels in plant-based diets in adult nutrition. This has led to direct assessment by protein scoring, which requires agreement about the scoring pattern and the requirement levels.

The adult lysine requirement

Values for lysine intakes are shown in Table 1. Food-supply data indicate >4-fold differences in *per capita* lysine supplies between countries consuming high and low levels of animal protein. Similarly, food-intake data for the UK show approximately 2-fold differences between omnivores and vegetarians. Whether these differences are important and influence the BV of plant proteins in human nutrition depends on the MD for lysine and all other amino acids, and this is a complex, unresolved and controversial question.

A number of proposed values for the lysine requirement are shown in Table 1. The value from the FAO/WHO/United Nations University (1985) report was derived from an earlier FAO/WHO (1973) report, but the later report was the first to publish age-specific amino acid requirement and scoring patterns. The IAA requirement values fell markedly with age for all amino acids, from over 50 % of total protein requirement in infants to only 16 % in adults. Based on this adult value (12 mg/kg per d) all diets listed in Table 1, which include some of the poorest in the world, are adequate. Thus, after 1985, apart from digestibility, protein quality ceased to be an issue in the nutrition of adults. However, the FAO/WHO (1991) report on protein-quality evaluation rejected the FAO/WHO/United Nations University (1985) adult value (and IAA requirement values for older schoolchildren) as flawed, was unable to identify any other appropriate adult scoring pattern, and proposed that the scoring pattern for the preschool child should be utilized for older schoolchildren and for adults as a strict interim measure. This value (58 mg/kg per d) was five times higher than the adult value of FAO/WHO/United Nations University (1985), and judged against this value food supplies in many developing countries are lysine deficient (see Pellett, 1996), as is the food intake of the UK vegetarian community and the whole of India (see Table 1). In my view it was a mistake to adopt this preschool pattern for older children and adults. As discussed elsewhere (Millward, 1994) this value was derived from studies (only partly reported and not peer reviewed) in children who had been malnourished and, as judged by the N accretion rates, may have been still replenishing lean tissue. This would account at least in part for the high values.

Young *et al.* (1989) derived a requirement on theoretical grounds with some support from stable-isotope studies (30 mg/kg per d), and these values have been the subject of considerable debate (see Millward, 1994). Finally, Ball at Guelph and Pencharz at Toronto have published what are referred to as the Toronto break point studies in which a requirement of 43 mg/kg was identified (Zello *et al.* 1993; Duncan *et al.* 1996). These values are discussed later.

Clearly, these values vary considerably, and judgement about the adequacy of the various intakes shown in Table 1 will depend on the choice of requirement value. This requires consideration to be given to the question of why it seems so difficult to decide on a value for lysine, or any other amino acid requirement.

Difficulties in defining amino acid requirements

Recently, it has been argued in detail (Millward, 1998b) that it is an inherently difficult problem to define the dietary requirements of human adults for IAA for three major reasons. The first of these reasons is adaptation, i.e. a variable MD for amino acids which is set by the habitual intake. The second reason is methodology, with no entirely satisfactory practical N- or amino acid-balance method or other measure of dietary adequacy. The third reason is lack of quantifiable unequivocal metabolic indicators of adequacy which can validate balance measurements. These issues will only be briefly mentioned here.

Nature of the metabolic demand for amino acids and protein

As reviewed recently (Millward, 1998b), the obligatory MD for dietary protein is to provide amino acid precursors for growth and maintenance, i.e. for an expansion of body protein and other N-containing material, and for the replacement of amino acids irreversibly transformed for whatever reason. The growth component of the MD is straightforward and determined by the growth rate and amino acid pattern of tissue protein deposited. However, as already discussed, growth needs are low at all ages after early infancy, with maintenance dominating the MD, and the problem in understanding the MD of maintenance is a major reason for the difficulty in defining the requirement. It can be shown that the MD for amino acids is best understood in terms of a two-component model; one component is obligatory, the other adaptive. The limited human data relating to the obligatory MD are consistent with extensive animal data showing the maintenance amino acid pattern to differ from that for growth, with lower levels of lysine and leucine in the maintenance pattern (Millward, 1997). The effect of this finding is that the rate-limiting amino acids in dietary proteins can differ between maintenance and growth, the example derived from rat experiments being that lysine limits wheat for growth but not for maintenance.

The adaptive component is best understood as an increased rate of oxidation of many of the IAA in response to levels in the habitual diet above minimal needs, which occurs independently of acute protein intake during post-absorptive and postprandial states of the daily feed-fast cycle at rates in excess of obligatory consumption (see Fig. 1), and which adapts only slowly to a change in intake. The implication of this increase is that the increasing catabolic losses with increasing protein intake represent an actual MD generated in response to the presence of protein in the diet at levels in excess of minimal metabolic needs. The impact of this component on the overall MD is difficult to predict due to a complex interaction between habitual protein intake (amount and quality) and (a) the

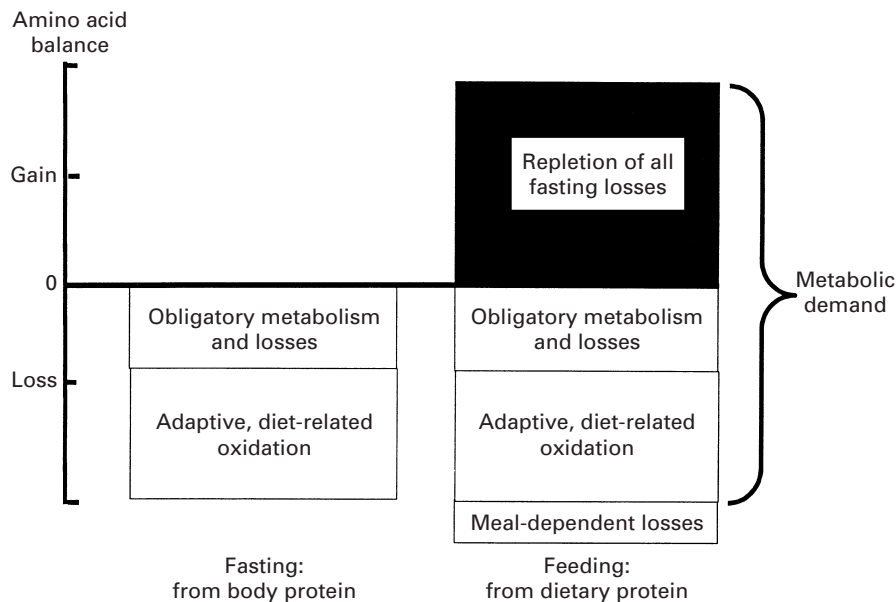


Fig. 1. Adaptive and obligatory metabolic demand for amino acids throughout the fasting and feeding cycle. During fasting, body protein provides for both obligatory and adaptive demands. During feeding, the diet provides for obligatory and adaptive demands, for repletion of tissue protein lost during fasting, and for any additional losses due to inefficiency of utilization. The adaptive components vary markedly with dietary intake (see Price *et al.* 1994) and change only slowly with intake (see Quevedo *et al.* 1994).

extent of post-absorptive oxidation rates and consequent losses of tissue protein to fuel the MD, (b) the extent of postprandial oxidation rates and tissue repletion and (c) the recycling of those IAA, such as lysine and threonine, with pool sizes large enough to allow this to occur. Although the time required for complete adaptation is not fully understood, it would appear to involve weeks and possibly months.

The practical implication of this adaptation component is that any attempt to measure the requirement in terms of the adequacy of any intake will depend on the completeness of adaptation to that intake.

Methodological difficulties

The second difficulty is methodology, with no entirely satisfactory practical N- or amino acid-balance method, or other measure of dietary adequacy. Following several critical reviews of N-balance studies (Hegsted, 1976; Young, 1986) and with the proliferation of stable-isotope studies, N-balance studies have not only become less fashionable, but have been rejected as of little value, or even performed and published but ignored when results conflict with those from stable-isotope studies (for example, see Marchini *et al.* 1993). In fact, close analysis of stable-isotope methodologies indicates that they are by no means free from difficulties. They are usually extremely complicated to interpret, so that few investigators fully understand their limitations, and as a result they can be frankly misleading. This is discussed extensively elsewhere (Millward & Rivers, 1988; Millward *et al.* 1991; Fuller & Garlick, 1994; Waterlow, 1996; Millward, 1986b), and one example serves to illustrate the difficulty.

This example is the studies of lysine requirements based on the indicator oxidation method (Zello *et al.* 1993; Duncan *et al.* 1996). This is an apparently simple approach in which subjects receive meals with decreasing amounts of lysine during which the oxidation of another amino acid is measured, in this case infused [$1\text{-}^{13}\text{C}$]phenylalanine. The principle is that when the lysine intake is inadequate to support protein deposition amino acids are diverted into oxidation, and this is indicated by increased phenylalanine oxidation. Thus, a plot of phenylalanine oxidation v. lysine intake reveals a two-component curve with a break point which defines the intake below which net protein synthesis in the postprandial state is compromised by lack of lysine. At this intake, identified in these studies as either 37 mg lysine/kg body weight per d (Zello *et al.* 1993) or 45 mg lysine/kg body weight per d (Duncan *et al.* 1996), amino acid oxidation increases. This approach derives from studies in growing animals where the requirement is relatively simply defined as accretion of protein. When adopted for human subjects to assess maintenance needs the assumption is made that there is a direct relationship between overall daily balance and maximum net deposition during feeding. Whilst even this assumption is in fact quite complex, for such studies to be of value what is reported as amino acid oxidation rate needs to be a true reflection of the actual rate. In fact, in these studies the phenylalanine oxidation rates were extremely low, of the order of 2–3 $\mu\text{mol/kg}$ body weight per h in subjects consuming approximately twenty times this amount of phenylalanine and tyrosine. The reason for this low value is an incorrect calculation method. The $^{13}\text{CO}_2$ production rate was converted into phenylalanine oxidation from the enrichment of plasma [^{13}C]phenylalanine rather than [^{13}C]tyrosine, the actual precursor for $^{13}\text{CO}_2$. In

the first paper (Zello *et al.* 1993) the authors quote values from which tyrosine enrichment can be calculated; I have done this calculation, and obtained a more realistic measure of total phenylalanine plus tyrosine oxidation. In fact, this error does not alter the break point other than to indicate a somewhat higher value, closer to 50 mg/kg body weight per d as far as can be calculated from these (admittedly mean and approximate) values.

Further inspection of these studies seems to indicate a fatal flaw in the design. The subjects received generous amounts of lysine at 60 mg/kg body weight per d throughout the studies, apart from the infusion days when the lower lysine meals were fed. Given that the maintenance requirement includes an adaptive component, this design would have generated a high requirement for lysine, so that it is not at all surprising that the study indicates values of between 30 and 50 mg/kg body weight per d. This would also explain why in the second study (Duncan *et al.* 1996) with the same experimental design no effect of previous protein intake on the break point was observed, since with a lower protein intake (0.8 g/kg body weight per d v. 1.0 g/kg body weight per d used by Zello *et al.* 1993) the observed requirement was not lower (45 mg/kg body weight per d). Dietary supplementation with additional lysine (and tyrosine) before the feeding studies would have prevented any adaptive influence of a lower protein-lysine intake on lysine oxidation and requirements.

The implications of the Toronto requirement values for 65 kg adults (Zello *et al.* 1993; Duncan *et al.* 1996) are shown in Table 1. Two values are shown: the average values quoted by Duncan *et al.* (1996) for these two studies for mean (43 mg/kg body weight per d) and safe requirements (i.e. mean and 2 SD; 63 mg/kg body weight per d). Not only is the whole of India severely lysine deficient, but the intakes of the UK vegetarian population are only barely adequate (see Table 1), and this is a group much more likely to be conforming to the healthy diet guidelines, with lower rates of morbidity and mortality than omnivores. If the safe allowances were adopted, then the intakes of UK vegetarians would all be markedly below them. Such implications do point to the 'requirement' value measured in this way as having little practical value and not representing an actual measure of minimum requirements.

Indicators of adequacy

A lack of quantifiable unequivocal metabolic indicators of adequacy which can be used to validate balance measurements is the third major difficulty in identifying requirements. Protein turnover in rats varies markedly in response to the adequacy of protein intake (Waterlow *et al.* 1978; Jepson *et al.* 1988). However, careful studies in human subjects indicate that protein turnover is in fact insensitive to protein (Pacy *et al.* 1994) or amino acid intake over a wide range (Marchini *et al.* 1993; Zello *et al.* 1993).

One metabolic approach is to examine plasma amino acid responses to feeding. The idea is that on eating, amino acid concentrations will rise when meals supply adequate amounts, and will fall when the amino acid intake is inadequate to support postprandial protein deposition. There

is an extensive literature on such responses (see Young & El-Khoury, 1996). Such work clearly does afford some insight into dietary adequacy, although such studies are only indirect measures, and can only provide at best supportive evidence of balance studies. However, even these relatively simple measures are not entirely unambiguous. Meredith *et al.* (1986) reported lysine concentrations in the fed and fasted state in subjects receiving various levels of lysine for 2 weeks, and Young & El-Khoury (1996) recently quoted these findings in support of the lysine requirement in the Massachusetts Institute of Technology scoring pattern of Young *et al.* (1989). When expressed as the molar proportion the values indicate that lysine levels fall with feeding when intakes are between 30 and 60 mg/kg body weight per d. However, when expressed as simple concentrations the fall with feeding does not occur until much lower levels are fed, between 10 and 20 mg/kg body weight per d. Thus, even these simple measurements can be used to support a high or a low lysine requirement. Identifying requirements is not a straightforward matter.

A way forward

My own view is to take notice of a recent statement by Nevin Scrimshaw (1996): 'The potential for error and bias has led some to reject nitrogen balance data in their entirety. It is foolish to accept poor data or to fail to recognise the limitations of good data, but it is equally inappropriate to reject reliable data as long as limitations are recognised.'

On this basis my own approach is to revisit the reliable N-balance data, recalculate the balances on a per kg body weight basis taking account of a reasonable estimate of miscellaneous losses, 5 mg N/kg per d (see Millward & Roberts, 1996), and to use a logarithmic regression to estimate the intake for zero balance as a measure of minimum maintenance needs, as suggested by Hegsted (1963). Whilst the N-balance data reviewed by FAO/WHO (1973) are quite variable in their quality, there are robust data for lysine.

The study by Jones *et al.* (1956; Fig. 2) involved fourteen women studied using semi-synthetic diets in which 95 % of N came from pure amino acids and ammonium citrate at various levels in a total of over fifty different balances with adequate but not excessive energy (136–157 kJ (33–38 kcal)/kg per d). I have recalculated all the data on a per kg body weight basis, including 5 mg miscellaneous losses/kg per d, assuming balance varies as $\log_e$ lysine intake. I have also included the data for the control periods when 25–30 mg lysine was fed, which Hegsted (1963) did not include in his analysis of these data. In fact the zero-balance intercept is not markedly different when based only on the sub-maintenance periods or on the entire data set. The intercept indicates a requirement of 18.6 (95 % CI 14.1, 27.3) mg/kg. Since subsequent studies of longer (30 d) balance periods indicated balance could be achieved at 11 mg/kg (Clark *et al.* 1962), I would assume that the value shown in Fig. 2 is greater than the minimum value achievable with further adaptation. Thus, the mean value of 19 mg/kg is a reasonable estimate of the lysine requirement.

Is this a sensible value? In fact it is not inconsistent with animal data, and is possibly slightly higher, being equivalent

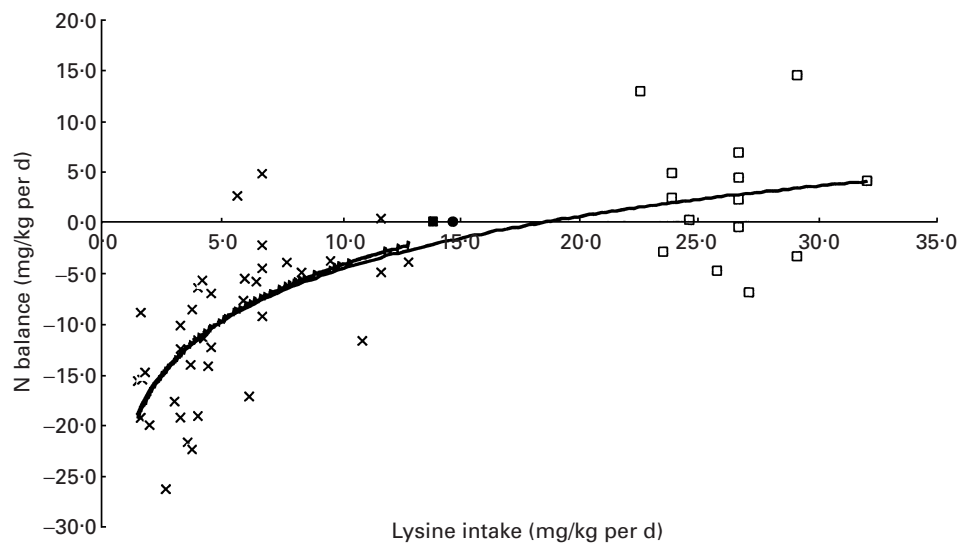


Fig. 2. Nitrogen balance studies of Jones *et al.* (1956). Values recalculated as balance per kg using individual body weights and adjusting for 5 mg N/kg additional losses. Values shown include sub-maintenance values (x) analysed by Hegsted (1963) and control values (□) also reported by Hegsted (1963). The regression of balance v. intake for all values is described by $\text{balance} = 7.45 \times \ln \text{intake} - 21.8 \text{ mg N/kg}$, with intake for zero balance at 18.6 mg lysine/kg. The regression of the sub-maintenance data is described by $\text{balance} = 7.857 \times \ln \text{intake} - 22.4 \text{ mg N/kg per d}$, with intake for zero balance at 17.2 mg lysine/kg. (■, ●), Values at zero balance are for maintenance lysine requirements calculated by two methods for the 40 kg pig (Fuller *et al.* 1989) scaled to 70 kg from values reported as $\text{kg body weight}^{0.75}$.

to $52 \text{ mg/kg body weight}^{0.75}$ compared with values ranging from 23 to $36 \text{ mg/kg body weight}^{0.75}$ for the rat and pig (Millward, 1997). Furthermore, this value has been validated. Two long-term studies with bread-based (wheat) diets have been reported. In the Minnesota study (Bolourchi *et al.* 1968) a diet in which 900 g/kg protein was supplied by wheat was fed for 50 d, supplying about 1 g protein/kg body weight and 18 mg lysine/kg body weight, with energy intakes designed to maintain weight against a high level of physical activity in the subjects (223 kJ (54 kcal)/kg); body weight, N balance and fitness were maintained. In the North Carolina study (Edwards *et al.* 1971) a lower-protein wheat and potato-based diet supplying 0.6–0.7 g protein and 24 mg lysine was fed for 60 d, and this induced maintenance of weight and N balance.

With reference to Table 1, this revised value for the lysine requirement indicates that for adults the low-meat or no-meat cereal-based diets in India and Sub-Saharan Africa supply adequate lysine, and of course UK vegetarians are more than adequately supplied.

Plant-based diets and children

The important issue is undoubtedly that of plant-based diets and children. My proposal (set out in Table 3) would be to adopt a factorial approach to calculating amino acid requirements. The starting point is the most recent values for protein requirements, which are based on a new estimate of maintenance and a growth requirement derived from known rates of tissue protein accretion adjusted for a 70% efficiency of utilization (see Dewey *et al.* 1996). A growth-requirement amino acid pattern can be derived from the

known composition of human tissues with minor adjustments for the S amino acids and tryptophan. As for the maintenance-requirement pattern, the N-balance data for the other IAA are not as robust as these lysine data, but reasonable estimates can be derived. Adjusted values for isoleucine, leucine, threonine and valine can be generated from the regression equations reported by Hegsted (1963), which are between 1.4 and 2.2 times the FAO/WHO/United Nations University (1985) values. For tryptophan the re-adjusted value is similar to the higher value reported by Rose (1957), which was adopted for the FAO/WHO/United Nations University (1985) requirement value, so this value is unchanged. For methionine plus cysteine the balance v. intake regression reported by Hegsted (1963) was so shallow that adjustment for unmeasured losses results in an approximately 3-fold increase, from 13 to $28 \text{ mg/kg body weight}$. This is an unrealistically high value. Since the obligatory oxidative loss for the S amino acids is $13.5 \text{ mg/kg body weight per d}$ (Millward & Rivers, 1988), it seems sensible to adopt an intake likely to deliver this value, i.e. $16 \text{ mg/kg body weight}$, as suggested by Young *et al.* (1989). For phenylalanine+tyrosine Hegsted (1963) only re-analysed a study based on phenylalanine in the presence of tyrosine. However, Tolbert & Watts (1963) subsequently reported requirement values of up to a maximum of $1184 \text{ mg phenylalanine/kg body weight per d}$, fed with no tyrosine and using positive N balance as their criterion. Thus, this value ($19.7 \text{ mg/kg body weight per d}$ for 60 kg body weight) has been adopted, i.e. 40% higher than the FAO/WHO/United Nations University (1985) value.

With these two patterns for growth and maintenance, age-dependent values for amino acid requirement patterns and

the corresponding scoring patterns (amino acid requirement/protein requirement) can be derived (Table 3) from the average requirement values for protein (Department of Health, 1991).

Validation of new requirement patterns for children

To validate these patterns a comparison can be made between predicted values for the BV of plant foods and the measured values (see Table 2). The amino acid contents of the plant food sources have been scored using the requirement pattern for a 3–6-month-old child. As discussed previously, the BV of potatoes, soyabean, various hybrid strains of maize, oats, rice, wheat and sorghum were measured relative to breast-milk. It is clear that in nearly every case the predicted value is similar to or less than the measured value, even though the scoring pattern used is that for younger children than those in the study. This value is appropriate, since they were exhibiting some catch-up and growing more rapidly than expected for their age. The fact that the scoring patterns do predict what is observed does provide a measure of validation.

Adequacy of plant-based diets in developing countries for children

The extent to which the low-lysine cereal-based diets of developing countries are adequate for children can be determined by scoring with these age-related scoring patterns (see Fig. 3). I have taken data recently published by Pellett (1996) for amino acid intakes in regions of India reported as preliminary data from the Indian National Food Intake Survey. These diets are probably as low in meat as any in the world. I have taken for the average value for India a diet in

which 77 % of protein was derived from cereal, supplying lysine at 39 mg/g protein (9.3 MJ (2254 kcal)/d, P:E 11.1 % energy); the highest-lysine diet, i.e. Tamil Nadhu, a diet with 64 % of protein from cereal, supplying lysine at 44 mg/g protein (7.8 MJ (1871 kcal)/d, P:E 9.7 % energy), and the lowest-lysine diet, i.e. West Bengal, based on 90 % of protein from cereal, supplying lysine at 39 mg/g protein (10 MJ (2426 kcal)/d, P:E 8.8 % energy). I have scored these diets with age-dependent scoring patterns for 6–12-month-old children and older children. I have also included the UK vegetarian group identified in the UK food intake survey (see Jackson & Margetts, 1993), with approximate calculations of their lysine intakes from the food group lysine data reported by Pellett (1996). Clearly, as might be expected, the score for the UK vegetarian diet is > 1 for all age-groups since, although meat-free, it did contain dairy products. The score for the Tamil Nadhu diet is ≥ 1 for all age-groups. The score for the mean value for India is 0.86 at 6–12 months, rising to 1 in the 2–5-year-old group. The score for the West Bengal diet is only 0.78 for the 6–12-month-old group and is inadequate for all preschool children (0.90 at 24–60 months). Clearly, the diets of all regions are adequate for adults. Thus, when weaned, Indian children in West Bengal will need either extra milk or pulses if the energy density of the diet does not allow them to increase intakes sufficiently to provide the extra intake. The main point is that the extent of the lysine deficit, when identified, is not very marked in any age-group.

Further evidence that would support these new scoring patterns comes from a more recent study by Graham *et al.* (1990) of young children recovering from malnutrition and growing at 2–3 g/kg body weight per d after the most rapid phase of catch-up growth had passed. They received the high-lysine maize variety (opaque-2, sugary-2; 43 mg lysine/g protein, which is similar to the values observed in the Tamil Nadhu diet) as their only energy, fat and protein source, which was as effective as casein in supporting both weight and height growth when supplemented with minerals and vitamins (Fig. 4).

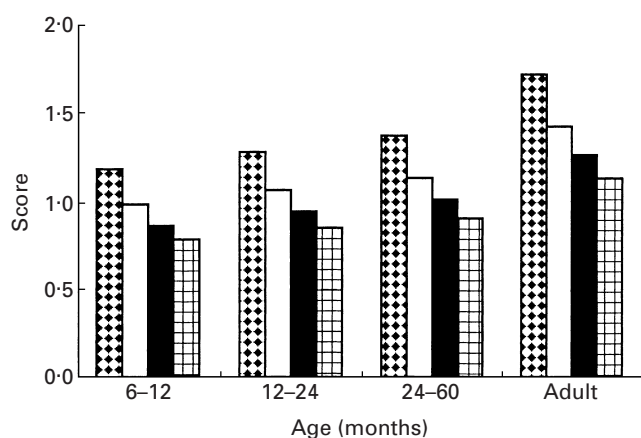


Fig. 3. Amino acid scores of plant-based diets in India and the UK for children and adults. Scores derived from intake data and scoring patterns shown in Table 3, with lysine as the limiting amino acid in each case. Intakes for the UK were calculated from the protein intakes of food groups reported by Jackson & Margetts (1994) and the lysine contents of food groups reported by Pellett (1996). Indian intake data were those reported by Pellett (1996). (▨), UK vegetarian; (□), Tamil Nadhu; (■), Indian (mean); (▤), West Bengal.

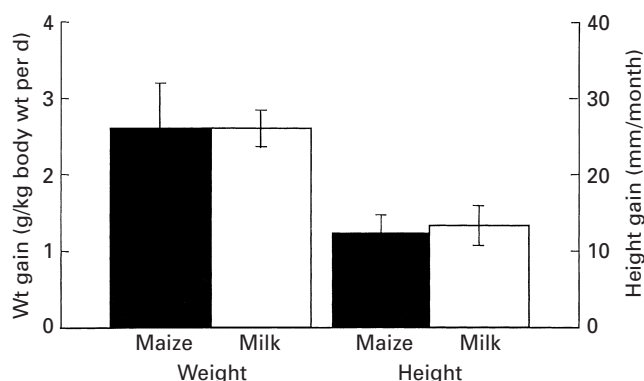


Fig. 4. Growth of recovering malnourished children on maize or milk protein. Recovering malnourished children aged 1–2 years fed on isonitrogenous diets supplying 0.42 MJ (100 kcal) metabolizable energy/kg per d, based on either milk protein or whole 'quality' maize (opaque-2, sugary-2 hybrid). (From Graham *et al.* 1990.)

Conclusions

Within the overall meat v. wheat debate there are several important nutritional issues relating to micronutrient and mineral nutrition which need to be addressed. However, in the present paper I have dealt with only one issue, i.e. the adequacy of plant food sources of protein and amino acids in the human diet. It is clear that human amino acid needs are complex and not entirely understood, and I think this is the main reason for the current debate about the magnitude of human amino acid requirements. It is also clear that there remains an inadequate body of reliable measures of such requirements. However, the current fashion for the complete rejection of the large body of N-balance data in adults and children is in my opinion a wholly inappropriate response, especially given that no adequate alternative data have yet emerged from stable-isotope studies. Assuming that lysine is the limiting amino acid in plant-based diets, we can be reasonably confident that the analysis reported here is accurate. On this basis it is clear that meat-free, largely plant-based diets available in developed countries can supply protein in the amount and quality adequate for all ages. In developing countries the protein issues relate mainly to low protein levels in some staples (taro, yams and cassava etc.), but cereal-based diets, especially those based on wheat and maize, supply protein levels considerably above the requirement level. Digestibility does present a problem for many mixed plant-based diets and some cereals (e.g. millet and sorghum), and this may be a major problem for children in the developing countries. More importantly, digestibility is less well understood in terms of colonic N homeostasis than commonly assumed, and more work is certainly required in this area. As for BV, this appears only relevant in infants and young children weaned on unsupplemented cereal diets, a practice which is already recognized as ill-advised. However, even in such cases, the extent of this problem is less marked than commonly assumed. This is not to underestimate the potential problems relating to poor micronutrient and mineral bioavailability of plant-based diets in infant nutrition. However, whatever the inadequacies of such diets, amino acid supply should not be used as the argument to promote increased intake of animal foods. Calls for lysine fortification and increased animal production to specifically supply more animal protein (for example, see Young & Pellett, 1990; Pellett, 1996) are certainly unjustified.

References

- Benevenga NJ, Gahl MJ, Crenshaw TD & Fink MD (1994) Protein and amino acid requirements for maintenance and growth of laboratory rats. *Journal of Nutrition* **124**, 451–453.
- Bingham SA (1997) Urine nitrogen as an independent validity measure of protein intake. *British Journal of Nutrition* **77**, 144–148.
- Bolourchi S, Friedeman CM & Mickelson O (1968) Wheat flour as a source of protein for adult human subjects. *American Journal of Clinical Nutrition* **21**, 827–835.
- Bressani R, Viteri F & Elias LG (1973) The usefulness of results in human beings of standardised rat assays. In *Protein in Human Nutrition*, pp. 293–316 [JWG Porter and BA Rolls, editors]. London: Academic Press.
- Cassidy A (1996) Physiological effects of phyto-oestrogens in relation to cancer and other human health risks. *Proceedings of the Nutrition Society* **55**, 399–417.
- Clark HE, Reitz LL, Vacharotayan TS & Mertz ET (1962) Effect of certain factors on nitrogen retention and lysine requirements of adult human subjects. *Journal of Nutrition* **78**, 173–178.
- Davis TA, Nguyen HV, Garcia-Bravo R, Fiorotto ML, Jackson EM, Lewis DS, Lee DR & Reeds PJ (1994) Amino acid composition of human milk is not unique. *Journal of Nutrition* **124**, 1126–1132.
- Department of Health (1991) *Dietary Reference Values for Food Energy and Nutrients for the United Kingdom. Report on Health and Social Subjects* no. 41. London: H. M. Stationery Office.
- Dewey KG, Beaton G, Fjeld C, Lonnerdal B & Reeds P (1996) Protein requirements of infants and children. *European Journal of Clinical Nutrition* **50**, Suppl. 1, S119–S147.
- Duncan AM, Ball RO & Pencharz PB (1996) Lysine requirement of adult males is not affected by decreasing dietary protein. *American Journal of Clinical Nutrition* **64**, 718–725.
- Edwards CH, Booker LK, Rumph CH, Wright WG, Ganapathy MS & Ganapathy S (1971) Utilization of wheat by adult man: nitrogen metabolism, plasma amino acids and lipids. *American Journal of Clinical Nutrition* **24**, 181–193.
- Food and Agriculture Organization/World Health Organization (1973) *Energy and Protein Requirements. Report of a Joint FAO/WHO ad hoc Expert Committee. WHO Technical Report Series* no. 522. Geneva: WHO.
- Food and Agriculture Organization/World Health Organization (1991) *Protein Quality Evaluation. Report of a Joint FAO/WHO Expert Consultation. FAO Food and Nutrition Paper* no. 51. Rome: FAO.
- Food and Agriculture Organization/World Health Organization/United Nations University (1985) *Energy and Protein Requirements. Report of a Joint Expert Consultation. WHO Technical Report Series* no. 724. Geneva: WHO.
- Fuller MF & Garlick PJ (1994) Human amino acid requirements: can the controversy be resolved? *Annual Review of Nutrition* **14**, 217–241.
- Fuller MF, McWilliam R, Wang TC & Giles LR (1989) The optimum dietary amino acid pattern for growing pigs; requirements for maintenance and for tissue protein accretion. *British Journal of Nutrition* **62**, 255–267.
- Fuller MF, Milne A, Harris CI, Reid TM & Keenan R (1994) Amino acid losses in ileostomy fluid on a protein-free diet. *American Journal of Clinical Nutrition* **59**, 70–73.
- Gibson NR, Ah-Sing E, Badaloo A, Forrester T, Jackson A & Millward DJ (1997) Transfer of ¹⁵N from urea to the circulating dispensable and indispensable amino acid pool in the human infant. *Proceedings of the Nutrition Society* **56**, 79A.
- Golden MHN (1997) Protein–energy interactions in the management of severe malnutrition. *Clinical Nutrition* **16**, Suppl. 1, 19–23.
- Graham GG, Lembcke J & Morales E (1990) Quality-protein maize as the sole source of dietary protein and fat for rapidly growing young children. *Pediatrics* **85**, 85–91.
- Graham GG, Morales E, Placko RP & Maclean WC (1997) Nutritive value of brown and black beans for infants and small children. *American Journal of Clinical Nutrition* **32**, 2362–2366.
- Health Education Authority (1996) *The Balance of Good Health*. London: Health Education Authority.
- Hegsted DM (1963) Variation in requirements of nutrients: amino acids. *Federation Proceedings* **22**, 1424–1430.
- Hegsted DM (1976) Balance studies. *Journal of Nutrition* **106**, 307–311.
- Jackson AA & Margetts BM (1993) Protein intakes in the adult population of the UK. *International Journal of Food Science and Nutrition* **44**, 95–104.

- Jepson MM, Bates PC & Millward DJ (1988) The role of insulin and thyroid hormones in the regulation of muscle growth and protein turnover in response to dietary protein. *British Journal of Nutrition* **59**, 397–415.
- Jones EM, Bauman CA & Reynolds MS (1956) Nitrogen balances in women maintained on various levels of lysine. *Journal of Nutrition* **60**, 549–559.
- MacFarlane G & Cummings JH (1991) The colonic flora, fermentation, and large bowel digestive function. In *The Large Intestine: Physiology, Pathophysiology and Disease*, pp. 51–92 [S Phillips, JH Pemberton and RG Shorter, editors]. New York: Raven Press.
- Maclean WC, De Romana GL, Placko RP & Graham GG (1981) Protein quality and digestibility of sorghum in pre school children: balance studies and plasma free amino acids. *Journal of Nutrition* **111**, 1928–1936.
- Malcolm LA (1970) Growth retardation in a New Guinea boarding school and its response to supplementary feeding. *British Journal of Nutrition* **24**, 297–305.
- Marchini JS, Cortiella J, Hiramatsu T, Chapman TE & Young VR (1993) Requirements for indispensable amino acids in adult humans: longer-term amino acid kinetic study with support for the adequacy of the Massachusetts Institute of Technology amino acid requirement pattern. *American Journal of Clinical Nutrition* **58**, 670–683.
- Meakins TS & Jackson AA (1996) Salvage of endogenous urea nitrogen enhances nitrogen balance in normal men consuming marginally inadequate protein diets. *Clinical Science* **90**, 215–225.
- Meredith CN, Wen Z-M, Bier DM, Matthews DE & Young VR (1986) Lysine kinetics at graded lysine levels in young men. *American Journal of Clinical Nutrition* **43**, 787–794.
- Millward DJ (1994) Can we define indispensable amino acid requirements and assess protein quality in adults? *Journal of Nutrition* **124**, 1509–1516.
- Millward DJ (1997) Human amino acid requirements. *Journal of Nutrition* **127**, 1842–1846.
- Millward DJ (1998a) Quality and utilization of plant proteins in human nutrition. In *Plant Proteins from European Crops: Food and Non-Food Applications*, pp. 169–176 [J Gueguen and Y Popineau, editors]. Berlin: Springer-Verlag.
- Millward DJ (1998b) Metabolic demands for amino acids and the human dietary requirement: Millward and Rivers (1988) revisited. *Journal of Nutrition* (In the Press).
- Millward DJ, Jackson AA, Price G & Rivers JPW (1989) Human amino acid and protein requirements: Current dilemmas and uncertainties. *Nutrition Research Reviews* **2**, 109–132.
- Millward DJ, Price G, Pacy PJH & Halliday D (1991) Whole body protein and amino acid turnover in man: what can we measure with confidence? *Proceedings of the Nutrition Society* **50**, 195–214.
- Millward DJ & Rivers JPW (1988) The nutritional role of indispensable amino acids and the metabolic basis for their requirements. *European Journal of Clinical Nutrition* **42**, 367–393.
- Millward DJ & Roberts SR (1996) Protein requirement of older individuals. *Nutrition Research Reviews* **9**, 67–88.
- Pacy PJ, Price GM, Halliday D, Quevedo MR & Millward DJ (1994) Nitrogen homeostasis in man: the diurnal responses of protein synthesis and degradation and amino acid oxidation to diets with increasing protein intakes. *Clinical Science* **86**, 103–116.
- Pellet PL (1996) World essential amino acid supply with special attention to South-East Asia. *Food and Nutrition Bulletin* **17**, 204–234.
- Price GM, Halliday D, Pacy PJ, Quevedo MR & Millward DJ (1994) Nitrogen homeostasis in man: 1. Influence of protein intake on the amplitude of diurnal cycling of body nitrogen. *Clinical Science* **86**, 91–102.
- Quevedo MR, Price GM, Halliday D, Pacy PJ & Millward DJ (1994) Nitrogen homeostasis in man: 3. Diurnal changes in nitrogen excretion, leucine oxidation and whole body leucine kinetics during a reduction from a high to a moderate protein intake. *Clinical Science* **86**, 185–193.
- Rose WC (1957) The amino acid requirements of adult man. *Nutrition Abstracts and Reviews* **27**, 631–647.
- Rosegrant MW (1999) Alternative futures for world cereal and meat consumption. *Proceedings of the Nutrition Society* **58**, 219–234.
- Scrimshaw NS (1996) Human protein requirements: a brief update. *Food and Nutrition Bulletin* **17**, 185–190.
- Tolbert B & Watts JH (1963) Phenylalanine requirements of women consuming a minimal tyrosine diet and the sparing effect of tyrosine on the phenylalanine requirement. *Journal of Nutrition* **80**, 111–117.
- Waterlow JC (1992) *Protein-Energy Malnutrition*. London: Edward Arnold.
- Waterlow JC (1996) The requirements of adult man for indispensable amino acids. *European Journal of Clinical Nutrition* **50**, Suppl.1, S151–S176.
- Waterlow JC, Garlick PJ & Millward DJ (1978) *Protein Turnover in Mammalian Tissues and the Whole Body*. Amsterdam: North Holland Elsevier.
- Young VR (1986) Nutritional balance studies: Indicators of human requirements or adaptive mechanisms. *Journal of Nutrition* **116**, 700–703.
- Young VR, Bier DM & Pellet PL (1989) A theoretical basis for increasing current estimates of the amino acid requirements in adult man with experimental support. *American Journal of Clinical Nutrition* **50**, 80–92.
- Young VR & El-Khoury AE (1996) Human amino acid requirements: a re-evaluation. *Food and Nutrition Bulletin* **17**, 191–203.
- Young VR, Fajardo L, Murray E, Rand WM & Scrimshaw NS (1975) Protein requirements of man: comparative nitrogen balance response within the submaintenance-to-maintenance range of intakes of wheat and beef proteins. *Journal of Nutrition* **105**, 534–544.
- Young VR & Pellet PL (1990) Current concepts concerning indispensable amino acid needs in adults and their implications for international nutrition planning. *Food and Nutrition Bulletin* **12**, 289–300.
- Young VR & Pellet PL (1994) Plant proteins in relation to human protein and amino acid nutrition. *American Journal of Clinical Nutrition* **59**, Suppl., 1203S–1212S.
- Zello GA, Pencharz PB & Ball RO (1993) Dietary lysine requirement of young adult males determined by oxidation of L-[1-¹³C]phenylalanine. *American Journal of Physiology* **264**, E677–E685.