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Chronic malnutrition: protein metabolism

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The topic of protein metabolism in chronic malnutrition is very broad and it seems appropriate to select a single issue which at the present is the subject of a controversy with widespread implications for policy and, hence, the health and well-being of large sectors of the population. The controversy centres on our understanding of the minimum requirements for protein and, therefore, deals with a number of fundamental issues. One of the questions raised, but not answered, by the controversy is the extent to which nutritional science can aspire to being value free.

Last year an article by Young & Pellett (1990) appeared in the *Food and Nutrition Bulletin*, with the title 'Current concepts concerning indispensable amino acid needs in adults and their implications for international nutrition planning'. The content of the paper had been promised since the most recent Food and Agriculture Organization (FAO)/World Health Organization (WHO)/United Nations University (UNU) expert consultation on protein and energy requirements (Food and Agriculture Organization/ World Health Organization/United Nations University, 1985) for Young had served notice of its advent for some years (Young & Bier, 1987). The recommendations for protein in the FAO/WHO/UNU document were based on the results of studies around the world using a standard N balance technique. There was general agreement on the desirability of developing more precise approaches to the definition of protein and amino acid requirements. Since 1985, Young's group in particular has published a series of papers in the scientific literature in which ^{13}C-labelled probes have been used to trace the fate of the carbon skeletons of the indispensable amino acids. The results of these studies led Young to the conclusion that the dietary requirement for the indispensable amino acids for normal healthy adults has been grossly underestimated by a factor of between two and three. Hence, the recommendations on the quality of dietary proteins which should be ingested to support requirements is unsafe. The conclusion has been reached that the requirements for essential amino acids cannot be met on diets which are based on cereals unless:
(a) at least 30% of the total protein is derived from animal sources, or
(b) at least 40% of the total protein is derived from animal sources plus pulses and soya beans (Young & Pellett, 1990).

If the pattern for essential amino acids proposed by Young (Young & Pellett, 1990) were to be used as the basis for assessing the needs for protein in the world there would

appear to be a widespread, global problem of protein deficiency. Were a problem of this kind to exist protein deficiency would become the major cause of chronic undernutrition and would, thereby, have the most profound implications for global food policy. A problem of this nature would in effect create a massive dependency of the developing world on the West, especially the USA, for the satisfaction of its food requirements. The implications for the environment would be horrific because it would only be possible to sustain a level of animal production which was adequate to satisfy human requirements at an extreme cost to the environment (Millward, 1992).

In 1989, a meeting was convened by the Codex Committee of the FAO to address the question of protein quality evaluation and in particular to explore the extent to which diets based entirely on vegetable-protein sources might be adequate. The Committee, *post hoc* given the status of an expert consultation by FAO/WHO, recognized the limitations of the protein efficiency ratio (PER) as the method for determining the quality of a protein for adults, the main problem being that the PER is based on the efficiency with which a protein can support the growth of rats. Therefore, the PER was rejected as the method of choice. The preferred approach for vegetable-protein products was considered to be the use of the amino acid scoring pattern with a correction for the true digestibility and/or the bioavailability of the limiting amino acid. The report from the meeting indicates some pressure to move towards a new amino acid scoring pattern for adults, based on the interpretation of the data collected by Young and colleagues. However, the evidence was not considered sufficiently strong to support this position fully. As a compromise interim measure it was agreed that the amino acid scoring pattern which was adopted for children in the Food and Agriculture Organization/World Health Organization/United Nations University (1985) report should be used for adults, until a clearer position has been reached.

This debate highlights a number of specific issues and problems related to our understanding of the requirements for amino acids and proteins and how these can best be determined in normal adults. Millward (1992) has discussed in some detail his criticisms of the limitations of the theoretical and methodological approach adopted by Young and colleagues. I would like to deal with more general considerations relating to our perceptions of dietary quality, essential and non-essential dietary components.

PROTEIN REQUIREMENTS: AMOUNT AND QUALITY

The classical approach to the assessment of protein requirements has been dominated by the general idea that proteins differ in quality based on their ability to provide sufficient amounts of an appropriate balance of the dietary essential amino acids. The basis of the assessment of adequacy in children has been the ability of the diet to support normal rates of weight gain and N balance. This approach has its limitations, but in general it is possible to determine increases in weight and positive N balance with a measure of reliability over reasonably short periods of time. For adults the difficulties are much greater. The end-points which have been used have been the ability of the diet to support N balance and maintain body weight. Neither of these are sensitive end-points, in part because of the ability of the physiological functioning of the body to adapt to a range of intakes, with the result that the definition of the point between adequacy and inadequacy can become very difficult to identify and define. If there is to be confidence in the results obtained, precise measurements have to be made over long periods of time, a very

difficult task to achieve in practice. For these reasons alternative approaches to the assessment of the N–amino acid–protein adequacy of the diet have been sought. In this respect the contribution made by Young's group is exceptional. By carrying out a complex series of studies with the use of stable isotopic probes they have provided invaluable data which quantify aspects of the complex interactions between diet and substrate flux. However, the interpretation of these results has to be coherent with the many observations made with the classical approach and any apparent differences have to be explained.

The average requirement for protein in the adult is in the region of 0·6 g/kg per d (Food and Agriculture Organization/World Health Organization/United Nations University, 1985), and it has been thought that less than 15% is required in the form of a balanced mixture of essential amino acids (Young & Bier, 1987). With over 80% of the dietary amino acids being provided as non-essential N most dietary protein sources would provide an adequate mix of amino acids. The level at which the adult recommended daily allowance (RDA) for protein was set did not change between the 1973 report and the 1985 report (Food and Agriculture Organization/World Health Organization, 1973; Food and Agriculture Organization/World Health Organization/United Nations University, 1985); however, there were differences in the justification for the values proposed. At the time of preparation of the 1973 report most of the data were based on studies carried out in healthy young men in the developed countries (Food and Agriculture Organization/World Health Organization, 1973). There were few data from the developing world, from groups at risk of deficiency or subsisting on diets of poor quality and low in protein. A major series of international comparative studies was carried out to determine the extent to which habitual diets of people living around the world were able to support adequate protein status (Rand *et al.* 1984). The studies were carried out under the auspices of the UNU to a standard protocol so that direct comparisons could be drawn between locations. The programme included both short-term and long-term studies and some studies were conducted in larger groups of people. The standard short-term method required the measurement of N balance at four different levels of protein intake with 10 d being allowed for equilibration to the new diet. Consideration can usefully be given to three sets of these data.

The study reported by Atinmo *et al.* (1988) from Nigeria is a good example of the results obtained with the adult protocol. The experimental diets were based on habitual foodstuffs, predominantly vegetable-based protein, and the four levels of protein intake were given in ascending order to half the group and in descending order to the other half. The derived values for protein requirements were 0·69 g protein/kg per d, with 0·75 g protein/kg per d covering the requirement for the group as a whole. One of the detailed points of interest was that there were significant differences in the N balance at three of the four levels of protein intake depending on whether the diets were taken in an ascending or a descending order. For the same level of protein intake N balance was more positive if the previous diet had a lower protein content than if the previous diet had a higher protein content. There appeared to be adaptation in N metabolism related to the earlier protein intake.

The second study was carried out in Chile in a group of children aged 8–10 years who received four levels of mixed protein from diets which were based on vegetable protein. For one level of intake (160 mg N/kg per d) a comparison was drawn with a milk and egg-based diet. Mean intake for satisfactory retention on the mixed-vegetable diet was

150 mg N/kg per d, suggesting a recommendation of 1·2 g protein/kg per d to cover 97·5% of the population. There was no difference in the balance achieved on the egg and milk diet compared with the vegetable-protein diet, except that on the vegetable diet there was a tendency to excrete more N in the stool, with a compensatory decrease in urinary N (Gattas *et al.* 1990).

In the third study the effects of marginal intakes of energy on protein metabolism and N balance in infants and young children were explored. Children grew at a normal rate on 419 kJ/kg per d and lost weight on 293 kJ/kg per d. However, the rate of loss was significantly greater during the first 3 d on the diet than at later times and by the end of a week on 293 kJ/kg per d there was a tendency towards weight stabilization. When 335 kJ/kg per d was given following a period on 419 kJ/kg per d weight was lost, whereas when 335 kJ/kg per d followed a week on 293 kJ/kg per d weight was gained at a rate not different to that seen on 419 kJ/kg per d (Jackson *et al.* 1983; Kennedy *et al.* 1990). Therefore, the response to a low intake of energy was determined in part by the previous diet. There was the suggestion of an adaptive response to an intake of 293 kJ/kg per d, allowing energy conservation, which carried through to the period on 335 kJ/kg per d. Stool frequency, which was about 1·2/d on 419 kJ/kg per d, declined to about 0·5/d on 293 kJ/kg per d and remained at 0·5/d on 335 kJ/kg per d, indicating that there was modulation of gastrointestinal function in the lower intakes. N balance was positive at all levels of dietary energy, from 293 to 419 kJ/kg per d, demonstrating that N balance can be defended in the face of overall negative energy balance.

Taken together the three studies show the effectiveness with which N can be retained by the body in normal health in response to low dietary energy or protein. Vegetable protein in the diet appears to be little different from animal protein except for higher faecal N, which can be effectively accommodated by a reduction in urinary N, to maintain overall balance. Changes in gastrointestinal function are associated with the achievement of N balance on marginal and low energy intakes.

ESSENTIAL AMINO ACIDS AND TOTAL NITROGEN INTAKE

There is a substantial body of evidence which shows that the dietary requirements for essential amino acids can be reduced if the total N content of the diet is increased. The nature of this relationship has never been adequately explained. A good example of the effect is seen in the study carried out in nine healthy young men, given three diets based on opaque-2 maize as the main source of protein (Kies & Fox, 1978). For the basal diet maize provided 4 g N/d (equivalent to 25 g protein/d, which is less than the requirement level). For the other two diets urea was added as a source of N equivalent to 4 and 8 g N/d, to give an overall intake of 8 and 12 g N/d (equivalent to 50 and 75 g protein respectively). The subjects tended to be in negative N balance on 4 g N/d ($-0·36$ g N/d) but significantly improved positive balance was achieved with the addition of 4 or 8 g urea-N (0·27 and 1·49 g N/d). These findings indicate that the requirement for essential amino acids can be reduced to very low levels provided the availability of non-essential N is generous. Furthermore, they imply that the N of the urea was made available in a metabolically useful form in significant amounts.

Our own approach has centred on defining the mechanisms of accommodation to low protein intakes and the conservation of N. It is known that of the daily urea production in the body a proportion is salvaged through the metabolic activity of the microflora of the

hind gut. The extent of the salvaging varies with the level of dietary protein and the overall metabolic activity of the host (Jackson *et al.* 1990; Danielsen & Jackson, 1992; Langran *et al.* 1992). It is possible to define three states of metabolism in relation to protein intake in normal adults, based on the rate at which urea is produced and salvaged. There are two break-points for urea kinetics as the dietary intake of protein decreases from adequate to inadequate: one at about an intake of 70 g protein/d (Fig. 1) and the other at about 35 g protein/d (Danielsen & Jackson, 1992). When the intake of protein exceeds 70 g/d (160 mg N/kg per d) about 70–75% of the urea produced is excreted in urine with 25–30% of the N being salvaged in the colon. Below 70 g protein/d down to 35 g protein/d (80 mg N/kg per d) a progressively greater proportion of the urea-N which is produced is salvaged with a lesser proportion being excreted. An intake of 35 g protein/d (80 mg N/kg per d) is the physiological minimum requirement and below this level N balance can no longer be maintained. The salvaged N appears to contribute to the maintenance of N balance and when the intake is 80 mg N/kg per d, salvage amounts to about 115 mg N/kg per d (on average 1·5 times intake; Langran *et al.* 1992). The inability to maintain N balance below 80 mg N/kg per d is associated with the salvage system failing (Danielsen & Jackson, 1992).

Vegetarians take a diet in which the protein is predominantly derived from plant sources and in the UK this provides about 57 g protein/d, significantly less than the consumption of omnivores, about 75 g/d (A. A. Jackson and B. Margetts, unpublished results). Therefore, as a matter of course, enhanced salvaging of urea-N would be found in vegetarians. In contrast, the habitual protein intake of omnivores is within a range where the salvage of urea-N is minimal. Recently, urea kinetics were measured in six free-living adult vegetarians on their habitual diet, using a single-dose method with no restrictions on life-style. For the group as a whole there was wide variability in the individual results for the proportion of urea production which was salvaged. However, when the rate of salvage was related to the protein intake, the results conformed with those obtained in metabolic studies under controlled conditions (Fig. 1). For each individual the intensity of urea-N salvage was appropriate for the intake of protein in the diet (A. A. Jackson and R. Bundy, unpublished results).

If these results are representative it can be concluded that the main determinant of the proportion of urea production which is salvaged is the dietary intake of protein. We have little direct evidence that the quality of the dietary protein exerts any influence. This would mean that urea salvage is determined primarily by the total food intake. The main determinant of food intake is energy intake which matches energy expenditure. Therefore, to a large extent the degree of urea salvage is determined by body size and activity, the two major determinants of energy expenditure.

The idea that the N from salvaged urea might have a functional use has not been widely accepted. The debate has hinged on assumptions about the form in which the urea-N might be returned to metabolism. It has generally been considered that ammonia generated from urea hydrolysis passes to the liver following absorption across the colonic mucosa. Hence, the ability to utilize urea-N would be determined by the ability of the liver to fix NH_3-N in a useful form: a strictly limited capability. Both *in vivo* and *in vitro* studies indicated that hepatic NH_3 is preferentially converted to urea with relatively little being fixed as amino acids (glutamate and serine–glycine exchange). The large amounts of NH_3 generated from urea hydrolysis are greatly in excess of the loads which the liver is normally considered capable of handling. Even if fixed innocuously the quantities of
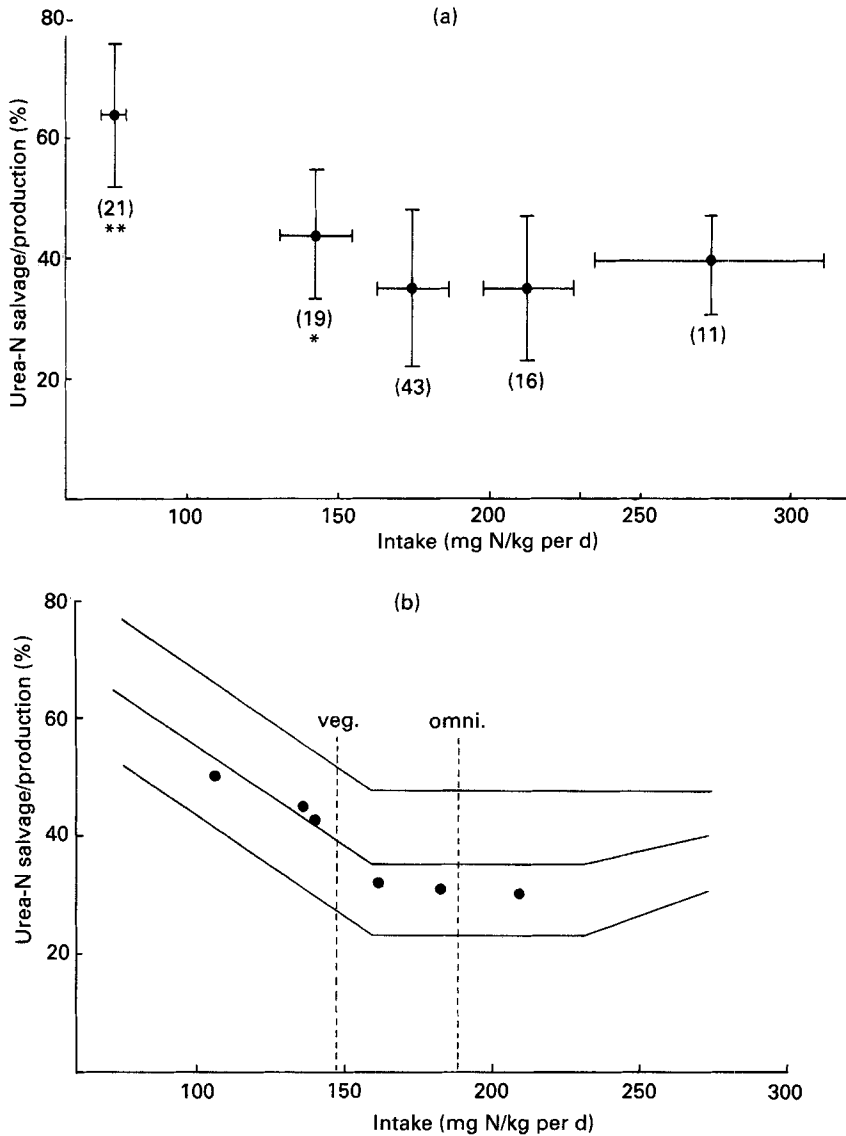
Fig. 1. A proportion of urea-nitrogen produced is salvaged in the lower bowel. An analysis of 100 studies in normal adults identifies a break-point at about 160 mg N/kg per d (1 g protein/kg per d). (a) Values for the proportion of urea-N production which is salvaged at different intakes of N. Points are the means and one standard deviation, represented by vertical bars, with the numbers of studies for each point shown in parentheses. Mean values were significantly different (ANOVA): $*P<0.05$, $**P<0.01$. (b) Values for individual measurements of salvage/production in six free-living normal adults who habitually consume a vegetarian diet. (●), Means and one standard deviation for salvage/production for the 100 studies; (– – –), weighted average intakes of protein from vegetarian (veg.) and omnivore (omni.) diets in the UK.

non-essential amino acids formed would generate a severe amino acid imbalance. When urea-N was placed directly into the colon 95% was absorbed very rapidly, with 5% as unchanged urea, 10% as NH_3 and the remaining 70–80% was fixed in the metabolic N pool (Moran & Jackson, 1990). It is presumed that the colonic microflora fix the NH_3 as

bacterial amino acids which are subsequently available for absorption. Both ^{15}N-labelled yeast protein and ^{15}N-labelled bacterial protein appear to be effectively absorbed across the colonic wall. Japanese workers have recovered label from urea in the plasma proteins of adults and children on low-protein diets and they have shown for both humans and rats that the label is present in all amino acids, essential and non-essential, including lysine (Tanaka *et al.* 1980). Papua New Guinea Highlanders have a traditional diet which is mainly vegetarian and low in protein (about 35 g/d). To achieve an adequate intake of protein would require a high level of activity and a concomitant increase in food and, hence, energy intake (Koishi, 1990).

It is possible to conclude that for protein there appears to be little difference between vegetarian and omnivore diets other than that associated with actual level of intake. This conclusion has very widespread implications and, therefore, has to be tested formally. The absolute intake of protein will be determined by the total food intake to satisfy the energy demand and will be determined by the size and activity of the individual. If the protein intake fails to reach 35 g/d then salvage and balance will not be maintained. Between 35 and 70 g protein/d the salvage system appears to play an integral role in satisfying the N requirements of the body. As yet we have insufficient information to say whether the requirements for protein, amino acids and N are best expressed in absolute terms or relative to either body weight or some component of lean body mass. Furthermore, we are unable to say whether at intakes above 70 g protein/d the salvage of urea-N makes a contribution of functional importance to metabolism.

In the standard approach to the assessment of N, amino acid and protein requirements the maintenance of external balance has been used as the primary determinant of adequacy. However, any general model of amino acid and protein metabolism has to take account of the nature and intensity of the internal exchange, first identified by Schoenheimer and quantified using the approaches developed by Waterlow. In this approach isotopically labelled probes of amino acids are used to measure the intensity with which amino acids move into and out of protein. Young's studies have provided detailed information on the turnover and oxidation of amino acids, based on the dilution of C and hydrogen labels in the plasma pool and the appearance of label in expired carbon dioxide. However, a full appreciation of the nature of the internal exchange also has to give consideration to the contribution made by the internal cycle through which urea-N is salvaged, and returned to the metabolic pool in a functionally useful form (Fig. 2).

CHILDHOOD; INFECTION AND CATCH-UP GROWTH

Food and Agriculture Organization/World Health Organization/United Nations University (1985) provides guidance on the intakes of energy and protein which would be required to maintain health in otherwise healthy individuals. As existence for many is normally characterized by periods of intercurrent infection and recovery from infection, arguments have been presented that recommendations should make appropriate allowance for the demands of recovery and a reasonable level of catch-up growth.

Tissue growth plays an important part in the determination of protein requirements in childhood. The relationship between energy requirements and protein requirements is non-linear because of the relatively high requirement for energy for maintenance with relatively little extra needed for growth, compared with the relatively low requirement of
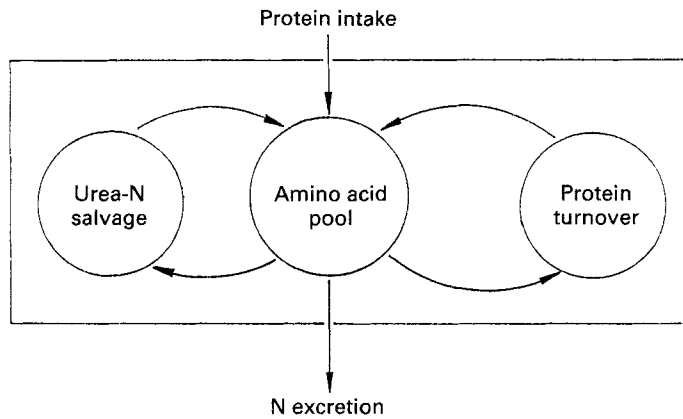
Protein intake

N excretion

Fig. 2. A model for assessing the nitrogen–amino acid–protein requirements of the diet has to take account of external balance as the relationship between protein intake and N excretion, but also has to embrace a consideration of the two major internal cycles: (1) the movement of amino acid into and from proteins, and (2) the salvage of urea-N through the metabolic activity of the colonic microflora.

protein for maintenance and relatively high requirement for growth. Therefore, the nature of the relationship between protein and energy requirements during growth will be determined by the rate of growth (a feature of the energy intake) and the composition of the tissue being deposited, in terms of the relative amounts of lean and adipose tissue. It can be shown that for a 1 year old, deposition of tissue comprising two-thirds adipose and one-third lean, rates of weight gain up to twenty times normal can be achieved on a diet in which the percentage of energy derived from protein (P:E%) is less than 3. In contrast, for tissue two-thirds lean and only one-third adipose, a diet with P:E% of 4 is required to support growth twice normal and nearly 10 to support growth at ten times normal (Jackson & Wootton, 1990). Most habitual diets have a P:E% between 10 and 15, human milk is about 7, and for some diets the P:E% may be as low as 8 (Waterlow, 1992).

Theoretical considerations for the losses of N associated with infections have suggested about 0·6 g protein/kg per d without diarrhoea and 0·9 g protein/kg per d with diarrhoea, e.g. 3 and 4·5 g protein/kg per d respectively for a 5 d period of illness. As in general the period of recovery is twice as long as the period of illness itself, over a recovery period of 10 d the retention would have to be 0·3 and 0·45 g protein/kg per d (equivalent to 1·5 and 2·25 g lean tissue/kg per d) (Schurch & Scrimshaw, 1992). If balanced tissue were being deposited during the recovery period (adipose–lean 50:50) rates of weight gain would be in the region of 3–5 g/kg per d. To determine the composition of the diet which could support these rates of weight gain a worst case situation can be assumed. The worst case situation in terms of protein would be an individual with a low requirement for energy (80% of the RDA) and the highest demand for protein (set at the safe level of intake) and an efficiency of protein deposition of about 60%. The energy required to deposit balanced tissue is about 21 kJ/g. On this basis the P:E% of the diet, which would be needed to support a range of rates of weight gains at different ages can be derived. It can be shown that under these circumstances the P:E% of the diet is inversely related to age. Substantial rates of weight gain can be supported on a diet with a P:E% of less than 10 in

Table 1. *An estimate of the proportion of the dietary energy which would have to be provided as protein (P:E%) in order to sustain increased rates of weight gain at different ages*

(It has been assumed that the energy requirement is at the lowest end of the range and the protein requirement is at the highest end of the range)

Rates of wt gain (g/kg per d) . . . Age (years)	P:E%				
	Normal	1	3	5	10
0·5	8·7	9·1	9·8	10·3	11·6
1–2	5·8	6·2	7·1	7·8	9·7
10	7·4	8·1	9·1	9·9	11·5
>60	20·3	19·6	18·7	18·2	17·9
Female, 80 kg					

childhood. In adults, because of the reduced requirements of energy for maintenance, an energy intake (and, hence, a level of total energy expenditure) of between 125 and 165 kJ/kg per d may be required with a P:E% between 10 and 15 to support even modest rates of weight gain (Table 1).

It may be concluded that dietary protein is not likely to be limiting for recovery from most diseased states in the young where the basal energy requirement is high, unless the diet is of very unusual composition. Protein is, if anything, more likely to be limiting in the older age-groups where a reduced level of activity, low energy requirements and low basal levels of energy expenditure result in low levels of food intake.

A full consideration of the adequacy of the diet in terms of protein requires that there is a clear understanding of each of the three aspects of the model of N, amino acid and protein metabolism: the external balance, and the two internal cycles, protein turnover and urea-N salvage. Young has drawn sweeping conclusions based on a partial understanding of only one component (Young & Pellett, 1990). He has overinterpreted the meaning of results from measurements of the flux of single amino acids and the rates of oxidation derived from measurements of the recovery of label from the C skeleton. The failure to take adequate account of the salvage system, especially for low levels of protein intake may well be of significance. Millward *et al.* (1991) have provided provisional evidence to suggest that at low levels of protein intake there may be disjunction between the fates of C and N labels during the process of catabolism of the amino acid, with relative preservation of N labels compared with C labels. It is likely that this difference can be completely accounted for by the difference in the metabolic fate of the label, exhalation in the breath or recovery through salvage in the colon.

REFERENCES

Atinmo, T., Mbofung, C. M. F., Egun, G. & Osotimehin, B. (1988). Nitrogen balance study in young Nigerian adult males using four levels of protein intake. *British Journal of Nutrition* **60**, 451–458.

Danielsen, M. & Jackson, A. A. (1992). Limits of adaptation to a diet low in protein in normal man. *Clinical Science* **83**, 103–108.

Food and Agriculture Organization/World Health Organization (1973). *Energy and Protein Requirements. Report of a Joint FAO/WHO ad hoc Expert Committee. Technical Report Series* no. 522. Geneva: WHO.

Food and Agriculture Organization/World Health Organization/United Nations University (1985). *Energy and Protein Requirements. Report of an Expert Consultation. Technical Report Series* no. 724. Geneva: WHO.

Gattas, V., Barrera, G. A., Riumallo, J. S. & Uauy, R. (1990). Protein-energy requirements of prepubertal school-age boys determined by using the nitrogen-balance response to a mixed-protein diet. *American Journal of Clinical Nutrition* **52,** 1037–1042.

Jackson, A. A., Doherty, J., deBenoist, M.-H., Hibbert, J. & Persaud, C. (1990). The effect of the level of dietary protein, carbohydrate and fat on urea kinetics in young children during rapid catch-up weight gain. *British Journal of Nutrition* **64,** 371–385.

Jackson, A. A., Golden, M. H. N., Byefield, R., Jahoor, F., Royes, J. & Soutter, L. (1983). Whole body protein turnover and nitrogen balance in young children around a maintenance intake of protein and energy. *Human Nutrition: Clinical Nutrition* **37**C, 433–446.

Jackson, A. A. & Wootton, S. A. (1990). The energy requirements for growth and catch-up growth. In *Activity, Energy Expenditure and Energy Requirements of Infants and Children*, pp. 185–214 [B. Schurch and N. S. Scrimshaw, editors]. Switzerland: International Dietary Energy Consultative Group.

Kennedy, N., Badaloo, V. & Jackson, A. A. (1990). Metabolic adaptation to marginal intakes of energy in young children. *British Journal of Nutrition* **63,** 145–154.

Kies, C. & Fox, H. M. (1978). Urea as a dietary supplement for humans. *Advances in Experimental Medicine and Biology* **105,** 103–118.

Koishi, H. (1990). Nutritional adaptation of Papua New Guinea Highlanders. *European Journal of Clinical Nutrition* **44,** 853–885.

Langran, M., Moran, B. J., Murphy, J. L. & Jackson, A. A. (1992). Adaptation to a diet low in protein: effect of complex carbohydrates upon urea kinetics in normal man. *Clinical Science* **82,** 191–198.

Millward, D. J., Price, G. M., Pacy, P. J. H. & Halliday, D. (1991). Whole-body protein and amino acid turnover in man: what can we measure with confidence? *Proceedings of the Nutrition Society* **50,** 197–216.

Millward, J. (1992). The influence of amino acid composition of protein on protein-energy interactions: the metabolic basis of amino acid requirements. In *Protein-energy Interactions* [B. Schurch and N. S. Scrimshaw, editors]. Switzerland: International Dietary Energy Consultative Group (In the Press).

Moran, B. J. & Jackson, A. A. (1990). The metabolism of ^{15}N-labelled urea in the functioning and defunctioned human colon. *Clinical Science* **79,** 253–258.

Rand, W. M., Uauy, R. & Scrimshaw, N. S. (1984). *Protein-energy Requirement Studies in Developing Countries: Results of International Research. United Nations University, Food and Nutrition Bulletin*, Supplement no. 10. Rome: UNU.

Schurch, B. & Scrimshaw, N. S. (1992). Protein-energy interactions. Switzerland: International Dietary Energy Consultative Group (In the Press).

Tanaka, N., Kubo, K., Shiraki, K., Koishi, H. & Yoshimura, H. (1980). A pilot study on protein metabolism in Papua New Guinea Highlanders. *Journal of Nutritional Science and Vitaminology* **26,** 247–259.

Waterlow, J. C. (1992). *Protein-energy Malnutrition*, p. 248. London: Edward Arnold.

Young, V. R. & Bier, D. M. (1987). Amino acid requirements in the adult human: how well do we know them? *Journal of Nutrition* **117,** 1484–1487.

Young, V. R. & Pellett, P. L. (1990). Current concepts concerning indispensable amino acid needs in adults and their implications for international nutrition planning. *Food and Nutrition Bulletin* **12,** 289–300.

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