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Maintenance protein requirements: the need for conceptual re-evaluation

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Protein requirements have been traditionally discussed in terms of the needs for accretion, (growth, pregnancy etc.), and the needs to allow whole-body protein or nitrogen equilibrium, usually described as maintenance. In the case of slow-growing organisms like man it is apparent from an examination of any set of requirement estimates (e.g. Food and Agriculture Organization/World Health Organization/United Nations University, 1985) that net accretion is seldom a large component and that maintenance requirements represent the major component of protein needs for most of life. Maintenance requirements are often the primary focus for any discussion of protein requirements for the simple reason that they are the least understood. The purpose of the present paper is to review the current problems associated with both estimating the magnitude and understanding the nature of maintenance protein requirements in man.

N BALANCE FINDINGS IN ADULTS AND THE INADEQUATE STATUS OF CURRENT REQUIREMENT VALUES

The current dilemmas and uncertainties associated with human amino acid and protein requirements have recently been reviewed (Millward *et al.* 1989), where it was emphasized that the main feature of the existing N balance findings is the extent of the disparity between individual studies. This disparity includes:

- (a) the shape of the N intake–N balance response curves,
- (b) the magnitude of the intake allowing apparent equilibrium,
- (c) the difficulty of demonstrating unequivocal differences in N balance responses between dietary proteins which differ in their indispensable amino acid content and hence protein score,
- (d) the existence of statistically identifiable cyclic patterns of N excretion and balance in some long-term balance studies but not in others,

- (e) the occurrence of enzyme changes thought to be pathophysiological in some long-term balance studies but not in others,
- (f) the existence of inverse correlations of urinary to faecal N excretion in some studies but not others.

The existence of such marked disparity in the published literature raises major problems to those trying to understand and construct a general description of the behaviour of the organism. To date the usual response has been to separate what is classed as 'representative' published findings from those studies which do not conform to the generally held view of what should occur and which must, therefore, be 'unrepresentative'. The challenge is greatest to those expert international consultations given the task of synthesizing a quantitative statement about needs and requirements. In the absence of any obvious explanation for the discrepancies it is not entirely surprising that somewhat arbitrary decisions can be made about data selection and aggregation by consultations in deriving numerical values for protein requirements. One case in point relates to the current values for the adult mean protein requirement. This derived in part from evaluation of a list of short-term balance studies conducted with single high-quality proteins. This list did not include all studies which had been published. The most notable omission was a series of short-term balance studies with egg protein in young male adults (107 individuals with 131 balance trials, far and away the largest series ever reported; Young *et al.* 1973) which if included would have resulted in a markedly lower mean requirement value. The average requirement for equilibrium was 0.46 g protein/kg (0.375–0.59, 95% confidence limits) compared with the 0.61 g protein/kg average of the studies listed in the report. (It is interesting to note that these confidence limits imply that some individuals achieved equilibrium on intakes close to the endogenous losses of 338 mg. This implies a biological value of 90% similar to the values reported by Nicol & Phillips (1976) in Nigerian farmers which is cited as indicating adaptation (Food and Agriculture Organization/World Health Organization/United Nations University, 1985). However, the subjects of the Young *et al.* (1973) study were young North American men.) Clearly this omission contributed to a considerable extent to the increase in the mean adult protein requirement over the previous (Food and Agriculture Organization/World Health Organization, 1973) value. Why this omission occurred has never been explained, but our point is that given the task of translating such a disparate mass of information into a single value it is to some extent not surprising that what appear to be arbitrary decisions are made.

There is no doubt that this concern for the adequacy of current requirement values is widespread (e.g. Beaton & Chery, 1988) and in many discussions increasing concern is being expressed about the methodological difficulty, particularly that of the N balance method. In a recent review from the perspective of considerable experience Young *et al.* (1989) summarize the major problems and identify technical problems relating to sensitivity, precision and errors associated with measurements of N intakes and losses. However, what is most important is the identification by these authors of a different class of problems, those which can be best described as 'model' problems. These later problems include the influence of extrinsic factors such as energy intake on N balance, the possibility that N equilibrium might be achieved at various states of protein dynamics, and the difficulty of validating the biological significance of apparent N balance. Thus, such criticisms of the N balance method imply that the established perception of the nature of protein requirements is inadequate. The problems are

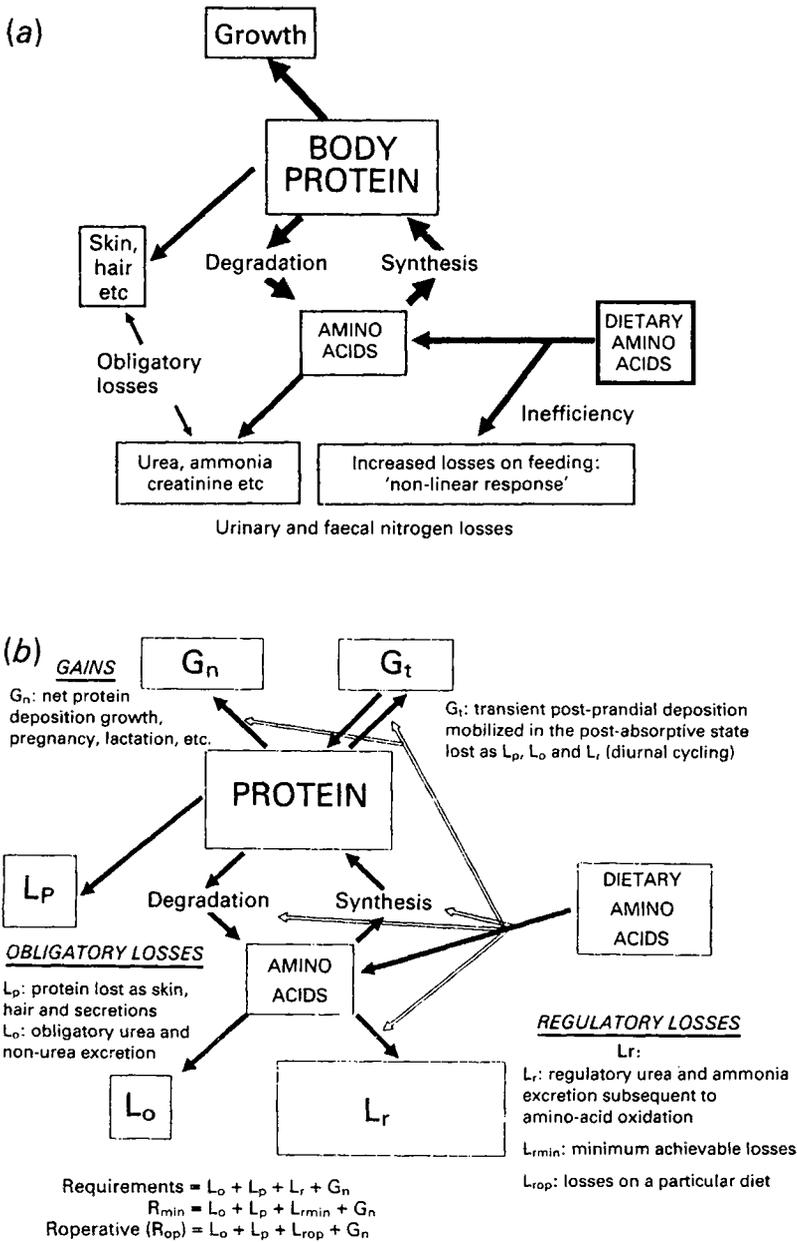


Fig. 1. Models for amino acid requirements. (a) Model which has been implied by most authors. Needs are viewed as the replacement of obligatory nitrogen losses and the provision for growth and any other net protein needs. The inefficiency of this provision, which is not adequately explained, is assumed to be fixed. (b) New model proposed by Millward & Rivers (1988). This model attempts to account for the apparent 'inefficiency' by identifying the factors which influence regulatory oxidative losses of amino acids. \rightarrow , Substrate flow. Dietary amino acids are also needed to exert a regulatory influence on several of these pathways of utilization \dashrightarrow . This is termed the anabolic drive.

'model' problems because they imply not only that regulation of N equilibrium is a complex response to extrinsic factors but an indicator other than balance could be the main criterion of dietary adequacy. This was the main purpose of the development of the new model described by Millward & Rivers (1988).

THE BIOLOGICAL BASIS OF THE NEED FOR DIETARY PROTEIN AND AMINO ACIDS

The model which seems to be implied by most current discussions of protein requirements is shown in Fig. 1(a). Dietary amino acids are needed to replace the obligatory N losses and to provide for growth and any other net protein needs, and this dietary provision is always inefficient. The current mean requirement of 0.6 g/kg implies an efficiency of only 60% in replacing the obligatory N losses of 0.34 g/kg. The key question is whether this inefficiency is fixed for an individual. If it is, then the requirement is fixed. If it is not fixed, then the requirement is variable. In fact this question cannot currently be answered unequivocally. However, a variable efficiency in response to extrinsic factors which are not yet sufficiently understood and consequently not always controlled for in balance studies is the most plausible explanation of the apparent disparity between the balance information referred to previously.

In fact there has been little discussion in the literature of this apparent inefficiency. Munro (1985) has described it as the 'non-linear response', explaining that for a high-quality protein the organism utilizes it with perfect efficiency to provide for obligatory needs until equilibrium is approached, at which point excretion increases to match any further intake. Thus, the curvature which causes the non-linearity is assumed to be an indication of the organism's less than perfect ability to sense equilibrium and adjust immediately. In fact this is an inadequate explanation since the shape of the N balance curve is quite variable between studies. As argued previously (Millward & Rivers, 1988; Millward *et al.* 1989) some reports indicate linear responses throughout the range: others show quite concave curves, with poor utilization at low intakes and better utilization near equilibrium. This variability in responses is also observed in studies examining the requirement for high-quality protein as well as studies examining the relative utilization (i.e. the biological value) of different proteins. Indeed because of the lack of reproducibility of balance studies it is extremely difficult to demonstrate consistent differences in human adults between N balance responses to proteins as disparate in their indispensable amino acid content as egg and wheat gluten (Millward *et al.* 1989). We can only conclude that it is due to factors which have not yet been identified.

After reviewing the large volume of balance information and considering what is known about amino acid and protein metabolism, Millward & Rivers (1988) came to the conclusion that the real problem is that the model within which we formulate requirements is simply inadequate and having decided this it was a relatively simple task to develop a new one (Fig. 1(b)).

The model attempts to explain the inefficiency of utilization of dietary protein by identifying two factors which result in oxidative losses of amino acids.

The first factor is the recognition that the organism does not tolerate high concentrations of most of the indispensable amino acids. If they are not deposited as protein after a meal they are oxidized by high-capacity, highly regulated oxidative pathways (L_r ,

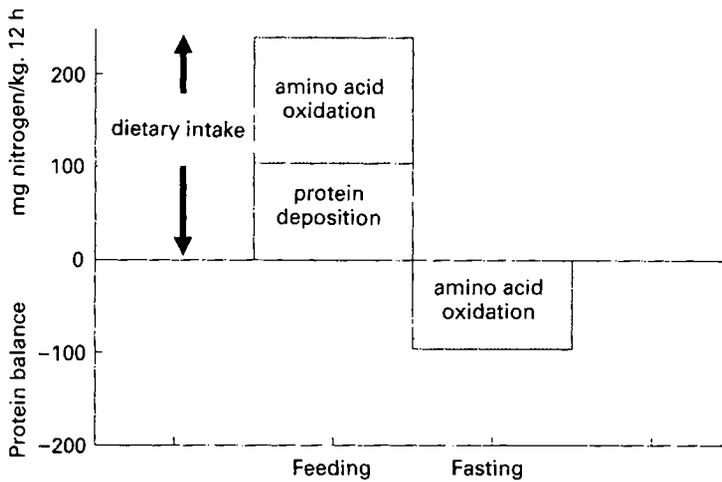


Fig. 2. Fate of dietary protein in adults over 24 h. Protein must be deposited in the fed state to match post-absorptive losses. The way in which losses are distributed between fed and post-absorptive states has an important implication for requirements.

regulatory losses). It is most likely that both the capacity and activity of these pathways are sensitive to acute and chronic dietary protein and possibly other influences.

The second factor which influences these losses is the periodicity of food intake, i.e. the diurnal pattern of feeding and fasting. The argument here is that body protein is lost in the post-absorptive (PA) state and that the extent of these losses is likely to be variable according to the extent to which changes in protein synthesis and degradation in response to fasting is conditioned by dietary protein intake.

The model also includes an additional concept, that of the anabolic drive, which is the idea that indispensable amino acids are needed to exert a regulatory influence on the organism and which will be discussed later.

MODEL VALIDATION

The model implies that requirements must be viewed in terms of meeting intrinsic needs (G_t , L_p and L_o) and needs determined by extrinsic influences on diurnal cycling and L_r . Thus, the efficiency of utilization of protein or an individual amino acid is determined by L_r which varies in a complex way in response to acute and chronic dietary influences and according to the periodicity of the intake. We have recently started to explore this latter aspect.

As shown in Fig. 2, in the context of a diurnal pattern of eating and fasting, protein must be deposited in the fed state to match PA losses if overall balance is to be maintained. A key question is the relative extent of oxidative losses in the fed and fasted state. There is no doubt that losses increase in the fed state and that these losses are variable with the diet (see Millward & Rivers, 1988). What is most important is whether PA losses are variable with the diet and the two possible situations are shown in Fig. 3.

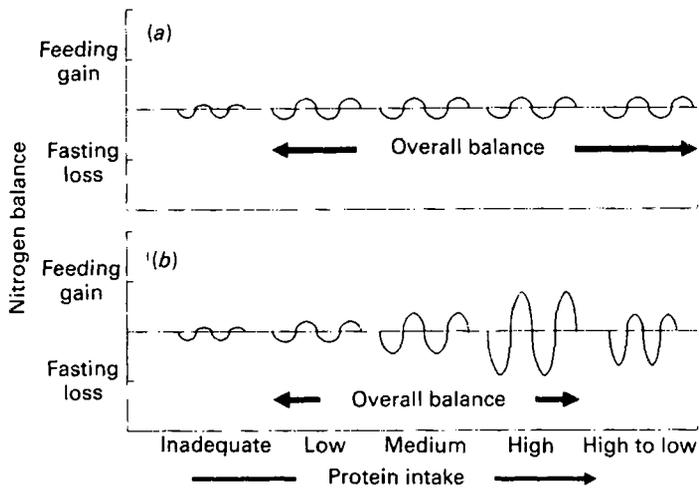


Fig. 3. Alternative models of diurnal cycling on varying protein intakes. The two models differ in whether post-absorptive losses are fixed or variable with the diet. (a) Constant low post-absorptive losses: as intake changes fed-state oxidation adjusts immediately, to allow a fixed low level of fed-state gain to balance post-absorptive losses. Balance is achieved at any intake above the minimum to allow sufficient fed-state gain, the amplitude of diurnal cycling should be constant and adjustment to a new level of protein intake should be immediate. (b) Variable post-absorptive losses: as intake increases both fed-state and post-absorptive oxidation increases requiring increasing fed-state gain for balance, i.e. increasing amplitude of diurnal cycling. Adjustment to a lower level protein intake is not immediate, given that the need for gain is determined by previous post-absorptive loss, with the actual time course of adjustment determined by the rate of change of amino acid oxidative capacity and whole-body protein turnover.

If there is a constant low PA loss, with no adaptation to the dietary protein intake, then for overall balance there should be a fixed low level of fed-state gain with fed-state oxidation adjusting immediately to eliminate all surplus intake. The amplitude of diurnal cycling should be constant and adjustment to new protein intake should be immediate.

However, from what we know (see Millward & Rivers, 1988), we can predict that L_r is likely to increase in both fed and PA state as dietary protein is increased and there are implications flowing from this if it is true. If there is induction of oxidative enzymes and a higher rate of protein turnover as protein intakes are increased and if such dietary conditioning persists for any appreciable time when the diet is changed then the pattern of response would be quite different. At low intakes there will be insufficient fed-state gain to balance PA losses so there will be negative balance. As intakes increase there will be increasing fed-state gains to balance increased PA losses. This means that fed-state deposition needed for balance will be determined by previous PA losses, in turn conditioned by previous intakes. Thus, after a period on a high intake a switch to a low intake should result in marked negative balance with insufficient fed-state gain for balance whilst the high PA losses persist. In other words adjustment to a new level of protein intake should take some time.

In an attempt to differentiate between these two models we have made measurements in individuals fed on increasing-protein diets. After 2 weeks on diets containing either

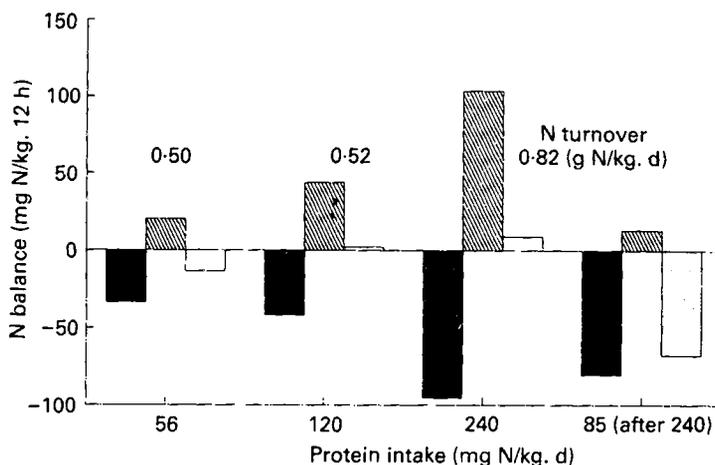


Fig. 4. Diurnal cycling in adults fed at varying intakes of protein. Nitrogen balances (12 h; from urinary N excretion corrected for the body urea pool changes) in normal adults measured over 48 h at the end of 2 weeks on the diets. Protein turnover calculated from $^{13}\text{CO}_2$ excretion during a constant infusion of L-[1- ^{13}C]leucine and the rate of N excretion. The amplitude of diurnal cycling increased with increased protein intake as did protein turnover. After an acute decrease in protein intake from the highest level, the persistence of high rates of oxidative losses meant that there was insufficient gain to balance the loss with a very marked negative balance (Price *et al.* 1990). (■), Fasting; (▨), feeding; (□), 24 h balance.

0.35 g/kg, (equal to the obligatory N losses (ONL)), 0.75 g/kg (current safe intakes) or 1.5 g/kg (twice the safe level), we have studied the amplitude of diurnal cycling over a 48 h period during alternate 12 h periods of feeding and fasting, measuring 12 h urinary N losses corrected for changes in the body urea pool, and whole-body protein turnover in the fed and fasting state with a combination of stable isotope methodologies (Price *et al.* 1990).

These preliminary results (Fig. 4) show that increasing the intake from the safe allowance of 0.75 g protein/kg per d to twice that level increased PA losses and post-prandial (PP) gains whilst approximate overall balance was maintained, and this was accompanied by an increase in the rate of whole-body protein turnover.

On the low intake there was insufficient PP gain to balance the PA loss with negative overall balance. When the diet was switched from a high to a low intake, the high rate of PA and PP loss persisted so that there was insufficient gain to balance the loss with a very marked negative balance.

These findings do suggest that our basic assumptions are accurate and that the central feature of our model is correct, that the requirement at any time is conditioned by previous intakes. What we still have to determine of course is how long this conditioning persists after the diet is changed.

In many ways of course the model has to be correct since it is built on the long-established phenomenon of labile protein reserves. In this new model labile protein reserves are seen as a manifestation of the kinetic and adaptive response to varying protein intakes.

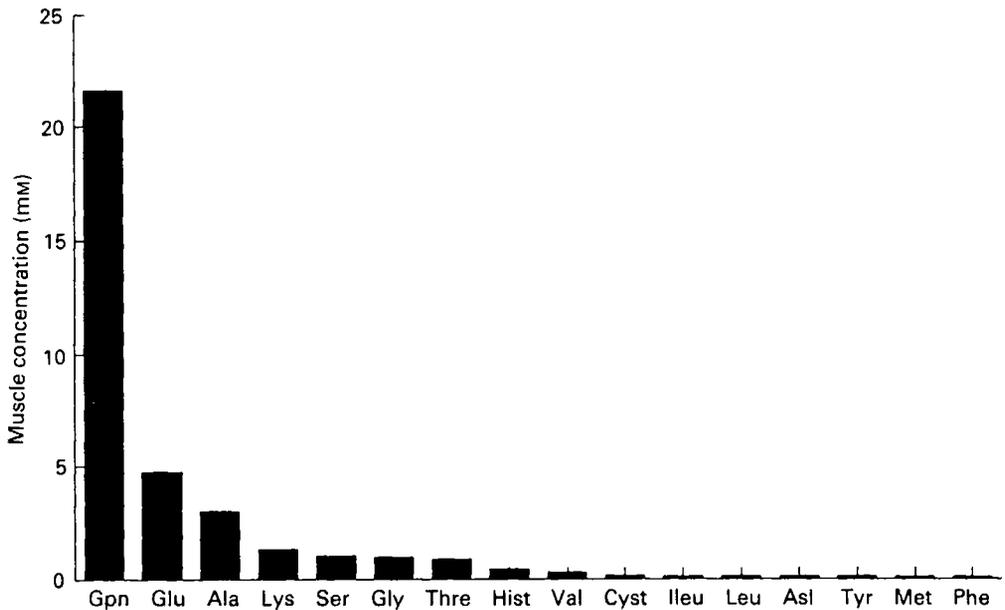


Fig. 5. Free amino acid concentrations in human muscle. Values from normal adult males (Millward *et al.* 1982).

INDISPENSABLE AMINO ACID REQUIREMENT VALUES

The idea that the indispensable amino acids are not tolerated by the organism is evident from an examination of the regulation of indispensable amino acid levels in body tissues. Fig. 5 shows the free amino acid concentrations in human muscle and it is obvious that whilst very high levels of some amino acids are tolerated, most indispensable amino acids are kept at very low concentrations. This happens because the oxidative enzymes do not allow the concentrations to rise (see Millward & Rivers, 1988). For the branched-chain amino acids (BCAA), for example, although they are the most abundant in protein the branched-chain α -keto acid dehydrogenase (*EC* 1.2.4.4), the rate-limiting enzyme in their oxidation, will not allow their concentration to rise and they are oxidized rapidly after feeding unless incorporated into protein. Judging by the responses to high levels of the BCAA and the aromatic amino acids in children with inborn errors of metabolism, these amino acids can be judged to be relatively toxic compared with the non-essential amino acids.

The significance of this in quantitative terms is that since we argue that the extent of L_r is variable according to both acute and chronic influences of the diet, then we can only unequivocally define the minimum value of L_r (L_{rmin}) and hence the minimum requirement (R_{min}). On any particular diet we can call the value of L_r observed the operative value (L_{rop}) with the requirement also defined as the operative requirement (R_{op}).

This leads to two questions: what do we know about the minimum value of L_r and hence the minimum requirement values and how does this relate to useful requirement values?

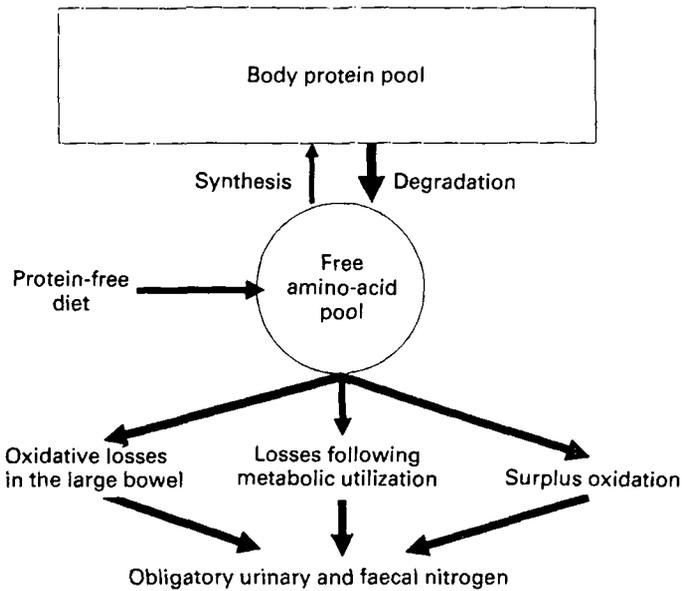


Fig. 6. Origin of the obligatory oxidative losses of amino acids. On a protein-free diet mobilization of body protein is assumed to occur to provide specific amino acids to replace those lost through oxidative losses in the lower gut, and through various metabolic pathways. Other amino acids released serve no particular need and will be oxidized since they cannot be re-utilized for protein synthesis. Thus, the overall pattern of losses will reflect tissue protein composition rather than the pattern of actual metabolic needs.

MINIMUM REQUIREMENT VALUES

According to what we know about the regulation of amino acid oxidation we can expect that minimum rates of oxidation should occur when intake is low and when tissue levels are low. The obvious circumstances when these conditions are found is on a protein-free diet, for example when the ONL are being measured.

In the adult in the absence of growth and discounting skin, hair and secretions, amino acids are required as precursors for various metabolites, neurotransmitters, hormones, cofactors and the like. In addition as discussed by Reeds (1990) amino acids are also required to replace those lost from the terminal ileum into the large bowel where amino-N is reabsorbed as ammonia but where much of the indispensable amino acid-carbon skeletons are lost during bacterial fermentation.

On a protein-free diet the source of amino acids for these needs is tissue protein which will be mobilized releasing a mixture of amino acids (Fig. 6). Those which serve some metabolic role will be used and eventually transformed to a nitrogenous end-product, those which are not will be oxidized directly since the now unbalanced mixture cannot be re-utilized for protein synthesis. If the relative pool sizes of the free amino acids is tightly controlled by the oxidative pathways then the overall rate of N excretion will be determined by the need for the rate-limiting amino acid, the indispensable amino acid with the highest ratio of metabolic need to concentration in protein. For all other amino acids their overall oxidative loss will be in excess of their metabolic need.

In fact it is an easy task to calculate the rate of loss of body indispensable amino acids which gives rise to the ONL, assuming they derive from tissue protein and assuming it has the composition of beef muscle. Millward & Rivers (1988) defined these losses as the obligatory oxidative losses (OOL). The problem is to determine which is the rate-limiting amino acid. There are two approaches to this particular problem. The first approach adopted by Millward & Rivers (1988) involved a comparison of the magnitude of the OOL with those of the Food and Agriculture Organization/World Health Organization/United Nations University (1985) adult requirement values for indispensable amino acids, in order to test the hypothesis that the Rose (1957) requirement values (which form the basis of the current adult values) were likely to be similar to minimum values (R_{\min}). It was assumed that the excess of dietary non-essential N (NEN) and energy in the original balance studies would have depressed oxidative losses. If this was the case then for the rate-limiting amino acid the magnitude of its OOL should be similar to that in the Rose (1957) pattern, whilst for all other amino acids the OOL should be greater. In fact the comparison indicated that the value for the sulphur-amino acids were similar in the two patterns, suggesting that the S-amino acids may be the rate-limiting amino acids driving the ONL. However, Young *et al.* (1989) rightly point out that there are several reasons why the Rose (1957) values may be inaccurate. If this is the case then it is not justifiable to compare the magnitude of the OOL with the Rose (1957) requirement values to identify the rate-limiting indispensable amino acid.

An alternative approach is to look at the experimental evidence arising out of either selective amino acid supplementation or depletion studies. Millward & Rivers (1988) reported that in several species the addition of methionine to a protein-free diet reduces N excretion. The most extensive studies of this kind in adult rats are those of Yoshida (see Yokogoshi & Yoshida, 1981) who evaluated the N balance responses to supplementing rats on protein-free diets and on rice- and wheat-protein diets with individual amino acids. They clearly showed that the pattern of the supplementation necessary for N balance was dominated by threonine and methionine with relatively little need for leucine and lysine. Fuller *et al.* (1989) held pigs at N equilibrium with a low-protein diet and examined the impact of depletion of individual indispensable amino acids on N balance. As shown in Fig. 7 the removal of the S-amino acids from the diets induces a negative N balance almost as great as a protein-free diet and greater than the removal of any other single amino acid. This indicates that for the pig the S-amino acids may be the rate-limiting amino acids driving the ONL, with threonine second limiting and with lysine and leucine appearing to be of less metabolic importance. These are very similar to the rat findings referred to above.

What this means is that R_{\min} for the S-amino acids may be close to the S-amino acid content of the OOL but for all other amino acids, particularly leucine and lysine, R_{\min} is less. In other words the pattern of the OOL must be quite different from the minimum requirement pattern. There is nothing remarkable about this since there is no *a priori* reason why the pattern of minimum requirements, which are metabolic needs, should be the same as that of tissue protein. In particular lysine and leucine which are of major importance for protein accretion due to their high concentrations in tissue protein appears to have less importance in the context of metabolic needs. This difference between maintenance and growth needs was clearly confirmed by Fuller *et al.* (1989) in his pig experiments. As suggested by the values in Fig. 7 and confirmed in subsequent experiments in these studies, maintenance requirements determined for the growing pig

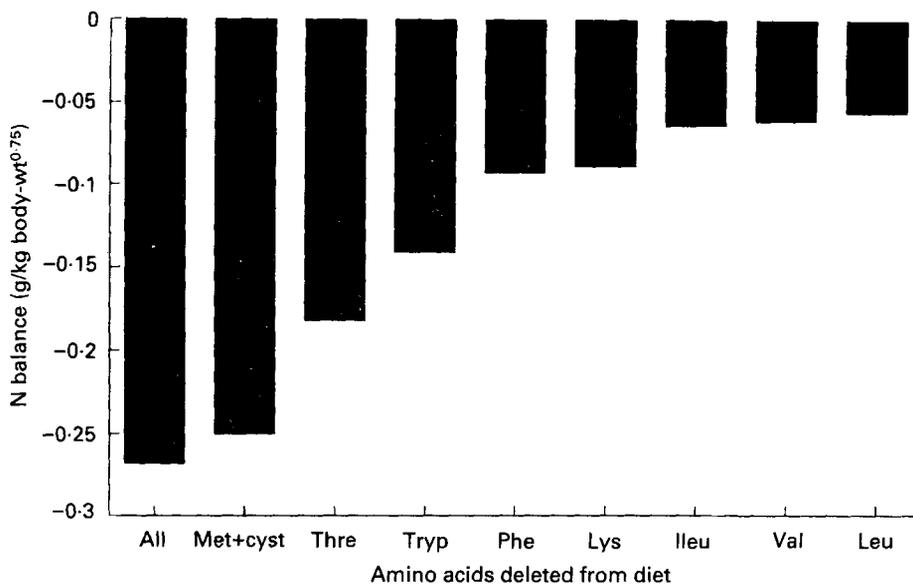


Fig. 7. Influence of deletion of individual amino acids from the diets of growing pigs. According to the extent of the negative nitrogen balance on removal of individual amino acids from the diets of pigs held at maintenance by a low-protein diet, sulphur-amino acids closely followed by threonine appear to be rate limiting for metabolic needs (Fuller *et al.* 1989).

held at protein equilibrium by a diet limited by indispensable amino acids have quite a different pattern from requirements for accretion (Fig. 8). The pattern of the OOL which Millward & Rivers (1988) calculated from the pattern of beef muscle bears a closer resemblance to the growth rather than the maintenance requirement. In particular leucine and lysine dominate the growth requirement and the OOL pattern whilst the S-amino acids and threonine dominate the maintenance requirements. Reeds (1990) has argued that in the latter case this reflects the predominance of the S-amino acids and threonine in the amino acid mixture lost from the terminal ileum into the large bowel.

Thus, the pattern of R_{\min} does appear to be different from the pattern of indispensable amino acids in tissues and the current requirement values for adults could be close to the values for the minimum requirements (i.e. where L_r is close to $L_{r\min}$). They were obtained in balance studies not with real proteins but with mixtures of amino acids containing very low levels of the eight indispensable amino acids with excess NEN. However, as already indicated, Young *et al.* (1989) believe that little attention should be paid to these values because of the inadequacy of the N-balance studies used by Rose (1957). These criticisms, however, cannot be levelled at Fuller *et al.* (1989) who also used this type of amino acid mixture in their recent pig experiments. As reviewed elsewhere (Millward & Rivers, 1988) there is ample evidence in the literature that NEN suppresses indispensable amino acid oxidation.

It should be clear from the previous discussion that the minimum maintenance

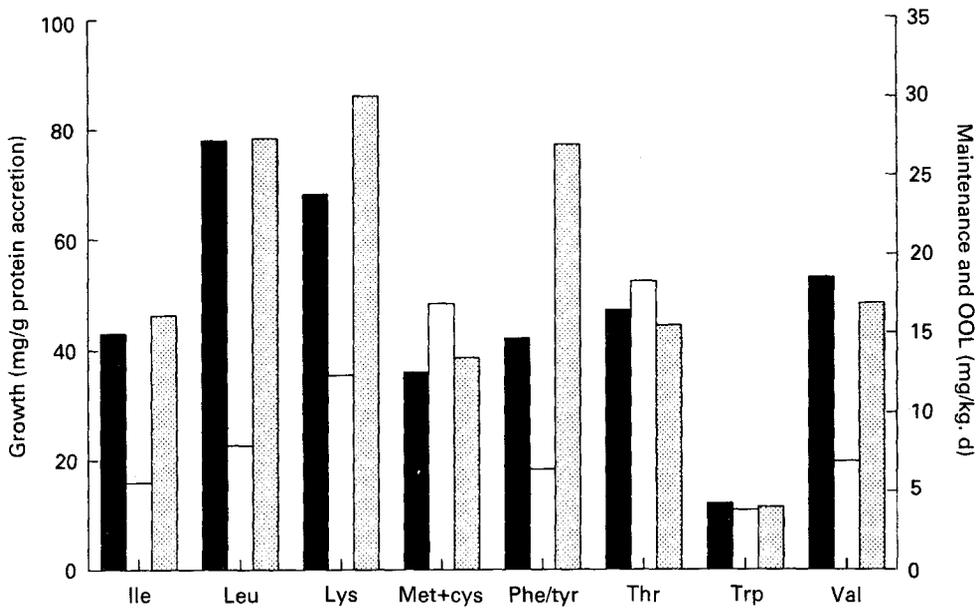


Fig. 8. Amino acid requirements for maintenance (\square) and growth (\blacksquare) of the pig and the pattern of obligatory oxidative losses (OOL; \boxtimes). Maintenance requirements are calculated for a 70 kg pig while growth requirements are for the accretion of 1 g protein (from Fuller *et al.* 1989). The OOL are the values calculated by Millward & Rivers (1988) based on the indispensable amino acids in an amount of beef muscle equivalent to the obligatory nitrogen losses. The requirement patterns for growth and the OOL correspond more closely than the maintenance requirement.

requirement pattern is quite different from the pattern of indispensable amino acids in tissue proteins (the pattern of the OOL). Because of this it can be in no way justifiable to take values for the OOL as the basis for a new requirement pattern as suggested recently by Young *et al.* (1989).

THE ANABOLIC DRIVE AND PRACTICAL REQUIREMENT VALUES

The most important issue, however, is whether these minimum requirement values are relevant to the needs of subjects on practical diets. Because L_{top} are increased on normal diets, the R_{op} are also increased. The extent of this increase may well be marked for an amino acid like leucine in which case the magnitude of R_{min} becomes irrelevant to needs on practical diets. It follows, therefore, that in order to make sensible statements about indispensable amino acid requirements which are of practical value, we need quite different criteria from those which we use to determine the minimum requirements. We believe that this is where the concept of the anabolic drive is useful.

The concept of the anabolic drive is simple. Amino acids are assumed to serve a dual role, acting as substrates for protein deposition and metabolism and exerting an important regulatory function by stimulation of anabolic processes. There is evidence that the indispensable amino acids are especially important for this latter role (see Millward, 1989*a,b*; Millward & Rivers, 1989).

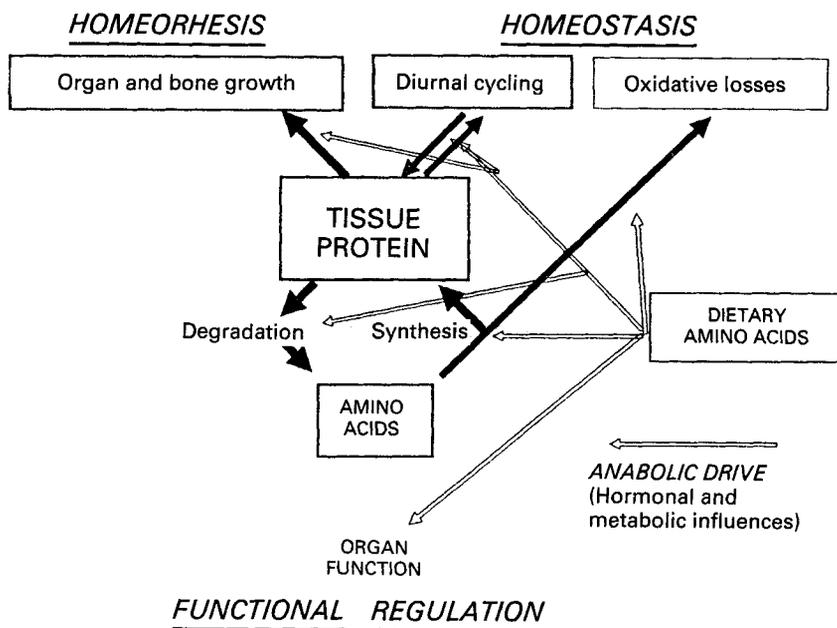


Fig. 9. The anabolic drive of dietary amino acids. The anabolic drive can be conceived as exerting homeostatic and homeorhetic influences as well as some functional regulation.

What we need, therefore, are indicators of the anabolic drive, i.e. functional responses of the organism which can be used to determine whether any particular level of apparent N or protein equilibrium is acceptable. Whilst this is a simple concept in practice it is by no means easy since, at present, the relationship between protein intakes and optimum organ function and health is poorly understood. As shown in Fig. 9 the anabolic drive can be conceived in terms of exerting homeostatic and homeorhetic influences as well as exerting some functional regulation. Dietary protein is well known to regulate the growth of skeletal muscle (Jepson *et al.* 1988) and in children growth in height may be another indicator of homeorhetic control by protein, at least according to the evidence assembled by Golden (1985).

In adults we might assess body composition and organ function. We know that muscle function is nutritionally sensitive (Lopes *et al.* 1982). There is also evidence that kidney function might be influenced adversely by excessive protein intake (Brenner *et al.* 1982) and this might define the upper safe limit of intake. Our main point is that without these indicators we cannot judge the efficacy of any level of protein intake.

CONCLUSION

It seems clear to us that understanding the regulation of N balance requires an understanding of both the regulation of amino acid oxidative pathways and the regulation of the diurnal cycling of body protein. We believe that there is much scope within this new

model for a re-investigation of the influence of the temporal pattern of food intake on N balance. It also remains to be established whether the considerable net deposition in the fed state on high-protein diets increases the requirements for indispensable amino acids such as lysine or whether diurnal fluctuations in the lysine free pool size could support such gains and losses on switching to a lysine-poor diet.

It also seems to us that in assessing protein requirements we need to be more willing to accept that the requirement for N balance may be variable. Because of this we must identify functional responses to the anabolic drive exerted by dietary protein with which we can assess independently the state of nutrition. Unless we are willing to take a new look at our conceptual basis for protein requirements we are unlikely to make any progress.

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