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Workshop on 'Protein and amino acid requirements and recommendations'

Methodological considerations

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What are we measuring?

In studies aimed at identifying protein and amino acid requirements a clear definition of objectives is important (Fereday *et al*. 1997; Millward *et al*. 1997). I define metabolic demands as what the organism needs, i.e. for maintenance plus additional needs for growth, pregnancy and lactation, efficiency of utilisation as the relationship between dietary intake and satisfaction of metabolic demands, and requirement as metabolic demands divided by efficiency of utilisation. We can measure requirement directly or calculate it after measuring metabolic demands and efficiency of utilisation.

How can we do it?

In terms of nutritional design, two types of studies are done.

Experimental studies with proteins enable measurements of protein requirements and protein quality (Food and Agriculture Organization/World Health Organization/United Nations University, 1985; Millward *et al*. 1989) to be made, which in turn enable (at least to some extent) amino acid requirements to be identified if the limiting amino acid is known (Millward *et al*. 2000*a*). The advantage of such studies is that protein and amino acid requirement values are used mainly to formulate food-protein-based diets so that such studies are most relevant. Indeed, it is important to recognise that information about requirements already exists in terms of our understanding of what individuals eat, i.e. the relationship between growth, maintenance, body composition, morbidity and mortality, and intake. Thus, requirements can be defined as intakes which maintain function.

The disadvantage of studies which only focus on protein-quality evaluation is that numerical values for requirements of amino acids can only be obtained for a few indisputably-limiting amino acids. Thus the second type of design involves experimental studies with amino acid mixtures which enable requirement values to be identified for all amino acids (Hegsted, 1963; Food and Agriculture Organization/World Health Organization, 1973; Meredith *et al*. 1986; Zello *et al*. 1992, 1995; El Khoury *et al*. 1994; Millward *et al*. 2000*a*). However such studies do raise the question as to whether utilisation efficiency is the same as that for whole protein (Collin-Vidal *et al*. 1994).

In terms of experimental design most studies involve measurements of either N losses (Food and Agriculture Organization/World Health Organization/United Nations University, 1985) or tracer-labelled-amino acid oxidation rates. The most useful studies are those which involve ^{13}C, enabling $^{13}CO_2$ excretion to be measured (Meredith *et al*. 1986; Young *et al*. 1987; Zello *et al*. 1992, 1995; Collin-Vidal *et al*. 1994; El Khoury *et al*. 1994; Fereday *et al*. 1997; Millward *et al*. 2000*a*). These studies permit factorial assessment of metabolic demands, efficiency of utilisation and requirement (Millward & Pacy, 1995; Fereday *et al*. 1997; Millward *et al*. 2000*a*), indirect assessment of requirement (Zello *et al*. 1992, 1995) or direct assessment of requirement in balance studies (Food and Agriculture Organization/World Health Organization/United Nations Organization, 1985; Meredith *et al*. 1986; El-Khoury *et al*. 1994). In addition, metabolic or functional responses in terms of plasma amino acids (Meredith *et al*. 1986), protein turnover (Collin-Vidal *et al*. 1994), or urea salvage (Badaloo *et al*. 1999), are also incorporated into such studies in an attempt to provide metabolic support for the physiological relevance of balance data. To date these latter measurements have been of limited use, and even plasma amino acid responses are not entirely unequivocal (Millward, 1999).

Finally, there have been attempts to predict requirement patterns from the composition of human tissues (Young *et al*. 1989; Young & El Khoury, 1995), but the legitimacy of this approach is debatable (Millward, 1990, 1994, 1997, 1998).

Limitations of nitrogen and ^{13}C balance studies

The main limitations are described in Table 1 and Table 2.

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Table 1. Potential problems relating to nitrogen balance methodology (for a detailed review, see Millward, 1998)

Problem	Reference
1. Imprecise	Wallace (1959), Forbes (1973)
2. Systematic errors:	
Intake overestimated, loss underestimated	Hegsted (1963)
Accounting for all losses	Calloway *et al.* (1971), Millward & Roberts (1996)
(a) Skin surface and secretions	Millward & Roberts (1996)
(b) Loss of N_2 gas	Costa *et al.* (1968)
(c) Expired ammonia	Calloway *et al.* (1971)
(d) Endogenous NO production gives urinary nitrate, faecal ammonia and nitrite	Kurzer & Calloway (1981)
(e) Changing size of the body urea pool	Price *et al.* (1994)
3. Non-linearity of the balance curve	Millward & Roberts (1996)
4. Design	
(a) Accounting for adaptation	Young (1986), Millward & Rivers (1988), Millward *et al.* (1990), Millward (1998)
(b) Dietary energy	Young (1986), Pellett & Young (1992)
(c) Amino acid mixtures *v.* proteins	Collin-Vidal *et al.* (1994)
5. Conceptual limitation	
(a) Functional significance of balance	Fuller & Garlick (1994)
(b) Adaptation (benign) or accommodation (adverse)	Young (1986), Young *et al.* (1987)

Table 2. Potential problems with ^{13}C isotopic measurements of amino acid oxidation rates and balance (for detailed reviews, see Millward, 1993, 1998)

1. Practical problems which are potentially soluble
   (a) Need for subject restriction and only short periods of study possible
   (b) The extent of isotope retention as bicarbonate
   (c) Measurement of CO_2 production rates
   (d) Variation in background tracer enrichment
   (e) Quantitative excretion of labelled C as CO_2
   (f) Oxidation rates as a tracer for overall N excretion influenced by mismatch of food and body tissue amino acid composition
   (g) Underestimation of losses due to non-CO_2 routes
   (h) Excessive positive balances in some studies
2. Model problems which are less tractable and possibly insoluble
   (i) Amount of 'tracer' excessive, and may influence balance
   (j) True precursor amino acid enrichment compared with measured value
   (k) Relationship between indirect measure of adequacy, e.g. postprandial oxidation rate, and overall balance unknown

General observations

Clearly there is a great deal we have to learn about the metabolism of amino acids, especially given the growing evidence for *de novo* synthesis of indispensable amino acids in nutritionally-significant amounts (Millward *et al*. 2000*b*). However, in my view progress will be more rapid if experimental studies build on rather than reject all existing knowledge, including the early N balance studies. Such knowledge is invaluable as a test of experimental data, which are often produced in very complex studies which few understand (Millward, 1999).

Protein requirements: Protein quality: Amino acid requirements: Balance studies

References

Badaloo A, Boyne M, Reade M, Persaud C, Forrester T, Millward DJ & Jackson A (1999) Dietary protein, growth and urea kinetics in severely malnourished children and during recovery. *Journal of Nutrition* **129**, 969–979.
Calloway DH, Odell ACF & Margen S (1971) Sweat and miscellaneous nitrogen losses in human balance studies. *Journal of Nutrition* **101**, 775–786.
Collin-Vidal C, Cayol M, Obled C, Ziegler F, Bommelaer G & Beaufrere B (1994) Leucine kinetics are different during feeding with whole protein or oligopeptides. *American Journal of Physiology* **267**, E907–E914.
Costa G, Ullrich I, Kantor F & Holland JF (1968) Production of elemental nitrogen by certain mammals including man. *Nature* **281**, 546–551.
El Khoury AE, Fukagawa NK, Sanchez M, Tsay RH, Gleason RE, Chapman TE & Young VR (1994) The 24-h pattern and rate of leucine oxidation, with particular reference to tracer estimates of leucine requirements in healthy adults. *American Journal of Clinical Nutrition* **59**, 1012–1020.
Fereday A, Gibson NR, Cox M, Pacy PJ & Millward DJ (1997) Protein requirements and ageing: metabolic demand and efficiency of utilization. *British Journal of Nutrition* **77**, 685–702.
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/United Nations University (1985) *Energy and Protein Requirements. Report of a Joint Expert Consultation. WHO Technical Report Series* no. 724. Geneva: WHO.
Forbes GB (1973) Another source of error in the metabolic balance method. *Nutrition Reviews* **31**, 297–300.
Fuller MF & Garlick PJ (1994) Human amino acid requirements: can the controversy be resolved? *Annual Review of Nutrition* **14**, 217–241.
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.
Kurzer MS & Calloway DH (1981) Nitrate and nitrogen metabolism in men. *American Journal of Clinical Nutrition* **34**, 1305–1313.
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 (1990) Amino acid requirements in adult man. *American Journal of Clinical Nutrition* **51**, 492–493.

Millward DJ (1993) Stable isotope tracer studies of amino acid balance and human indispensable amino acid requirements. *American Journal of Clinical Nutrition* **57**, 81–86.

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 (1998) Metabolic demands for amino acids and the human dietary requirement: Millward and Rivers (1988) revisited. *Journal of Nutrition* **128**, 2563S–2576S.

Millward DJ (1999) The nutritional value of plant based diets in relation to human amino acid and protein requirements. *Proceedings of the Nutrition Society* **58**, 249–260.

Millward DJ, Fereday A, Gibson NR & Pacy PJ (1997) Ageing, protein requirements and protein turnover. *American Journal of Clinical Nutrition* **66**, 774–786.

Millward DJ, Fereday A, Gibson NR & Pacy PJ (2000*a*) Human adult protein and amino acid requirements: [^{13}C-1] leucine balance evaluation of the efficiency of utilization and apparent requirements for wheat protein and lysine compared with milk protein in healthy adults. *American Journal of Clinical Nutrition* **72**, 112–121.

Millward DJ, Forrester T, Ah-Sing E, Yeboah N, Gibson N, Badaloo A, Boyne M, Reade M, Persaud C & Jackson A (2000*b*) The transfer of ^{15}N from urea to lysine in the human infant. *British Journal of Nutrition* **83**, 505–512.

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 & Roberts SR (1996) Protein requirement of older individuals. *Nutrition Research Reviews* **9**, 67–88.

Millward DJ & Pacy PJ (1995) Postprandial protein utilisation and protein quality assessment in man. *Clinical Science* **88**, 597–606.

Millward DJ, Price GM, Pacy PJH & Halliday D (1990) Maintenance protein requirements: the need for conceptual revaluation. *Proceedings of the Nutrition Society* **49**, 473–487.

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.

Pellett PL & Young VR (1992) The effects of energy on protein metabolism: a statistical evaluation. In *Protein-energy Interactions*, pp. 81–120 [N Scrimshaw and B Schurch, editors]. Lausanne: I/D/E/C/G.

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.

Wallace WM (1959) Nitrogen content of the body and its relation to retention and loss of nitrogen. *Federation Proceedings* **18**, 1125–1130.

Young VR (1986) Nutritional balance studies: Indicators of human requirements or adaptive mechanisms. *Journal of Nutrition* **116**, 700–703.

Young VR, Bier DM & Pellet PI (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 (1995) Can amino acid requirements for nutritional maintenance in adult humans be approximated from the amino acid composition of body mixed proteins? *Proceedings of the National Academy of Sciences USA* **921**, 300–304.

Young VR, Gucalp C, Rand WM, Matthews DE & Bier DM (1987) Leucine kinetics during three weeks at submaintenance-to maintenance intakes of leucine in men: adaptation and accommodation. *Human Nutrition Clinical Nutrition* **41** C, 1–18.

Zello GA, Pencharz PB & Ball RO (1992) Lysine requirement in young adult males. *American Journal of Physiology* **264**, E677–E685.

Zello GA, Wykes IJ, Ball RO & Pencharz PB (1995) Recent advances in methods of assessing dietary amino acid requirements for adult humans. *Journal of Nutrition* **125**, 2907–2915.