Optimal intakes of protein in the human diet

D. Joe Millward

Centre for Nutrition and Food Safety, School of Biological Sciences, University of Surrey, Guildford GU2 5XH, UK

For protein, progress is slow in defining quantifiable indicators of adequacy other than balance and growth. As far as current requirements are concerned, only in the case of infants and children is there any case for revision, and this change is to lower values. Such intakes would appear to be safe when consumed as milk formula. In pregnancy, notwithstanding the concern that deficiency may influence programming of disease in later life, there is little evidence of any increased need, and some evidence that increased intakes would pose a risk. For the elderly there is no evidence of an increased requirement or of benefit from increased intakes, except possibly for bone health. For adults, while we now know much more about metabolic adaptation to varying intakes, there would appear to be no case for a change in current recommendations. As far as risks and benefits of high intakes are concerned, there is now only a weak case for risk for renal function. For bone health the established views of risk of high protein intakes are not supported by newly-emerging data, with benefit indicated in the elderly. There is also circumstantial evidence for benefit on blood pressure and stroke mortality. With athletes there is little evidence of benefit of increased intakes in terms of performance, with older literature suggesting an adverse influence. Thus, given that a safe upper limit is currently defined as twice the reference nutrient intake, and that for individuals with high energy requirements this value (1·5 g/kg per d) is easily exceeded, there is a case for revising the definition of a safe upper limit.

Protein requirements: Pregnancy: Bone health: Renal function: Blood pressure

Historically, defining human protein requirements has been difficult and consequently controversial. The difficulty of addressing the question, 'requirements for what?', has precluded an entirely satisfactory answer to the major question of 'how much?'. The Food and Agriculture Organization/World Health Organization/United Nations University (FAO/WHO/UNU; 1985) report which forms the basis of current requirement values defined requirements for all ages as intakes which maintained N balance and which provided the additional protein needs during pregnancy and lactation, and during growth of infants and children. The issue of requirements 'for what?' was discussed only to a limited extent, i.e. the need to maintain protein turnover, with adaptive reductions in response to low intakes and to avoid deficiency diseases (listed as stunting, poor musculature and kwashiorkor-like pathology). The Department of Health (1991) report in essence adopted the magnitude and concepts behind the FAO/WHO/UNU (1985) values (with slight modifications to the allowances for pregnancy and lactation) with requirements 'for what?' limited to

'maintenance of a state of well-being'. The one new concept related to the beginning of a discussion, under the heading of 'Guidance on high intakes', of a safe upper limit. Thus, recognizing that in developed societies omnivores have protein intakes considerably in excess of the reference nutrient intake, and given the growing concern that excessive intakes of protein may be associated with health risks, it was deemed necessary to identify the concern and recommend caution. It was concluded that with insufficient information to enable a safe upper limit to be defined, it is probably prudent for adults to avoid protein intakes of more than twice the current reference dietary amount (i.e. 1·5 g protein/kg). In the present brief review it is intended to re-examine the recommendations of the Department of Health (1991) report in the context of moving from simple indicators of adequacy to more comprehensive measures, in order to see how much further we can go in defining optimal intakes of protein in the human diet. There is no intention of providing a comprehensive review, but to highlight the important areas where debate about benefits and cost is current.

Requirements for pregnancy

The protein requirements for pregnancy were limited to an additional 6 g protein/d to be added to the adult requirement through all stages of pregnancy. This amount was an estimation of the dietary requirement to allow for protein retention in the products of conception and in the maternal tissues associated with the birth of a 3·3 kg infant. The maintenance needs of the new tissue were assumed to be too small to require any additional needs.

No suggestion has been made about revising this recommendation. However, the possibility that protein intakes during pregnancy may require reconsideration has arisen out of Barker's work (see Barker, 1998) on the fetal origins of adult disease. Since rat studies have shown that a low-protein diet fed to the pregnant dam results in offspring with elevated blood pressure (Langley & Jackson, 1994), and since nutrition during human pregnancy has been shown to relate to blood pressure in children (at least as far as maternal haemoglobin status is concerned; Godfrey et al. 1994), there is concern that protein intakes during human pregnancy may be an important factor influencing birth weight and adult morbidity. Several recent reports from Barker's group (see Campbell et al. 1996; Godfrey et al. 1996, 1997) provide information on this relationship. In a prospective observational study of placental and birth weights and maternal diet, a high carbohydrate intake in early pregnancy and low intakes of dairy and meat protein in late pregnancy were associated with lower placental and birth weights (Godfrey et al. 1996). The relationship between diet in pregnancy and thinness at birth was investigated by Godfrey et al. (1997), who reported that women with a high intake of carbohydrate in early pregnancy and a low intake of dairy protein in late pregnancy tended to have infants that were thin at birth. In a follow-up study of men and women born 40 years ago whose mothers had taken part in a survey of diet in late pregnancy, diet in pregnancy was compared with the offspring's current blood pressure (Campbell et al. 1996). The authors report that low birth weight was related to increased adult blood pressure, but the dietary aspects of this relationship are complex, i.e. an apparent adverse influence of low-protein diets on blood pressure amongst mothers with intakes of animal protein less than 50 g. In fact, the data show that overall the protein : energy (P : E) value of the maternal diet was inversely related to birth weight, and this was especially strong for animal protein. Furthermore, while for individual nutrients there were no trends for any nutrient intake in relation to blood pressure, for animal protein intakes above 50 g/d there was a direct relationship between the P : E value of the animal protein and blood pressure. Clearly, such data require very careful interpretation. The reported protein intakes in these studies where apparent effects of protein intakes were observed are by no means low (e.g. 70–72 g/d and 100 g/d for the lower and upper quartiles in the study of Godfrey et al. 1996). Furthermore, there is clear evidence of risk associated with increased protein intakes through supplementation. Thus, re-analysis of the sixteen published trials of protein supplementation in pregnancy showed that protein supplements lowered birth weight (Rush, 1989). Thus, in my view, great care is required to avoid over-interpreting the results in a way which suggested benefit from increased protein intakes in pregnancy.

Optimum protein intakes for infants

Protein requirements for infants have been calculated in a semi-factorial way from a maintenance requirement based on a few short-term N-balance studies, choosing the highest value of 120 mg N/kg per d, and a growth requirement calculated from estimated rates of N accretion to which additions were made of 50 % to account for day-to-day variability in growth and for an inefficiency of dietary utilization (assumed to be 70 %). It was pointed out that when this factorial calculation of mean intakes was converted into a safe intake (+ 2 SD) the value was considerably higher than the average intakes of breast-fed infants, being, in fact similar to the intakes of formula-fed infants (Beaton & Cheery, 1988). Since infants of healthy, well-nourished mothers having protein intakes provided as breast-milk exhibit satisfactory growth rates, it must be assumed that there are negligible prevalence rates of inadequate protein intakes. This finding indicates that the average breast-milk protein intake is effectively the safe level, and should be similar to, rather than lower than, the factorially-derived safe intake. As a result, the suggestion was made that the requirement estimates for infants should be lowered (Beaton & Cheery, 1988).

This suggestion has been further discussed recently in an attempt to better define the actual biological demand for protein in relation to infant growth rates (Dewey et al. 1996a). New estimates of requirements have been derived from a lower maintenance requirement (90 mg N/kg per d) and growth rates not adjusted for day-to-day variability. Such values result in safe levels which now relate closely to breast-milk intakes, although it must be said that the establishment of breast-milk intakes as the model for validating a safe intake level is not without influence on the process of deriving a biological requirement.

This is an important issue, raising critical questions about appropriate protein contents of infant formula and protein needs at weaning. The current and revised values, and intakes from breast-milk are shown in Fig. 1.

In the Department of Health (1991) report it was recognized that the intake of protein and N from breast-milk is lower relative to the energy intake than at later stages of life, even though this is the age of most rapid growth, with N in breast-milk being utilized with unusual efficiency, for reasons poorly understood. Thus, because of the enhanced efficiency of utilization, it would be unwise to use breast-milk protein intakes as the reference values against which intakes of other infant food proteins can be compared. The implication was that protein in formulas or mixed feeds would be utilized at a sufficiently lower efficiency than breast-milk to make the higher formula or mixed feed intakes equivalent to those from breast-milk.

Since 1991 more data have been reported which allow a re-examination of this issue, and these data have led Dewey et al. (1996a) to propose that requirements for infants should be lowered because there is no advantage of the higher intakes from formula and no possible risk.
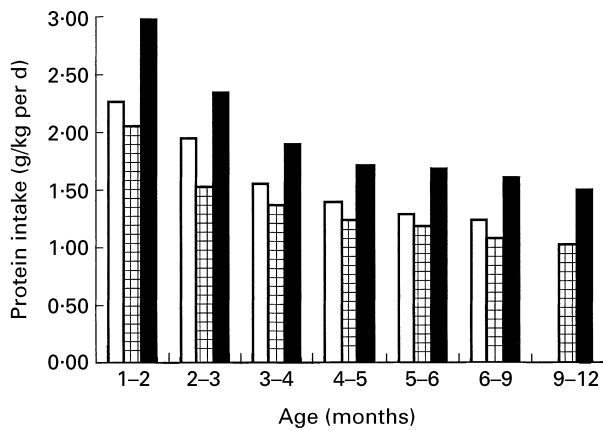
Protein intakes from breast milk, current requirements and proposals for revised requirements during the first year of life. (□), Intakes recalculated from Dewey et al. (1996) as means of the two values for intake at the start and end of each period. (■), Reference nutrient intake values calculated from maintenance and growth values as in Food and Agriculture Organization/World Health Organiza-

Dewey's own work (see Dewey, 1997) has examined the specific questions of whether breast-milk protein intakes are adequate, and whether the higher formula intakes confer any advantage, especially given concern for their safety in relation to acid–base balance and renal solute load. When comparisons are made between rates of growth of breast-milk- and formula-fed infants during the first year of life (the DARLING study; Dewey et al. 1992, 1993; Heinig et al. 1993), the higher energy and protein intakes with formula feeding are associated with greater weight and fat-free mass gains than those observed in breast-fed infants. However, Dewey (1997) argues that in these and other studies (for example, see Cohen et al. 1995) the main difference is in weight and fatness, with length-growth differences being much less marked. Furthermore, any benefit of excess weight gain should be considered in the context of potential risk, since not only do breast-fed infants do better in terms of outcomes such as immune function and behavioural development, but also in the DARLING study the higher protein intake at 6–9 months was significantly related to higher morbidity (Dewey et al. 1995). Indeed, according to Dewey et al. (1995) 'the reduction in morbidity associated with breast-feeding is of sufficient magnitude to be of public health significance'. It would appear, therefore, that there is no evidence of any functional advantage to the more rapid growth of formula-fed infants.

Foman et al. (1995) have examined the question of the safe P : E value for infant formulas and the relative adequacy of the lower protein intakes of breast-milk in a study of infants fed on milk-based formulas of low P : E compared with a usual-formula control group and a previously-studied large reference group. In the experimental group protein intakes were similar to those from breast-milk (slightly lower in the youngest infants), and while gains in weight and concentrations of serum albumin did not differ from those of the two control groups, gains in length were

significantly lower. They concluded that the P : E values for the experimental formula diet (3·7 g/MJ (1·56 g/100 kcal) decreasing to 3·0 g/MJ (1·25 g/100 kcal)) were below the safe level. However, with such small differences in growth and no difference in albumin 'we suspect that the safe protein-energy ratio of infant formulas lies closer to the ratios fed to the experimental group than to the ratio in currently marketed milk-based formulas (approximately 5·0 g/MJ (2·10 g/100 kcal))'.

The question of the adequacy of breast-milk protein intakes has also been examined in an intervention study (Dewey et al. 1996b) in which comparisons were made between a cohort exclusively breast-fed for 6 months and a breast-fed group supplemented with 20 % extra protein at 4–6 months. Multiple between-group comparisons indicated that weight and length gain were not different, and that after controlling for energy, protein intake did not influence growth. Dewey (1997) comments that this finding was consistent with that of the DARLING study, in that within the breast-fed cohort protein intake was not associated with weight or length growth after controlling for energy intake (Heinig et al. 1993).

On the basis of these and other arguments Dewey et al. (1996b) present a strong case in support of the breast-fed infant as a model for requirements. The arguments include benefit in terms of outcome indicators such as immune function and behavioural development (which may or may not be a function of the protein level), and evidence that the protein intake is not marginal (weight or length growth during breast-feeding not limited by protein), and is safe (morbidity inversely related to protein intake). Dewey (1997) argues that the evolutionary compromise theory proposed by Fomon (1991), that breast milk provides an intake which is a compromise between maximizing infant growth and health and minimizing maternal lean tissue depletion, does not hold up to scrutiny. Thus, the advantage to the mother of the lower milk-protein production against a dietary background of generous protein intakes would be too small to influence outcome and selection. It does seem, therefore, that breast-milk provides a protein or N intake at the level of the biological requirement with no obvious benefit and possible risk from higher intakes.

The elderly

Current protein requirements for the elderly derive from FAO/WHO/UNU (1985) recommendations of no change with age in adults i.e. average 0·6 g/kg and safe allowance 0·75 g/kg. Although concern has been expressed that protein requirements for the elderly may be increased, our review of N-balance data, none of which is entirely satisfactory, indicates little reason for any revision (Millward & Roberts, 1996). Two important studies have addressed the issue of whether or not variation in protein intake towards higher intakes within the normal range is beneficial in elderly subjects consuming self-selected diets.

Munro et al. (1987) reported measurements of dietary intakes, plasma proteins and arm muscle area for 691 men and women aged 60–98 years consuming on average 1·04 g protein/kg. Only 12–15 % of subjects had protein intakes

below 0·8 g/kg (possibly reflecting inadequacies of 3 d records), but clearly overt 'protein deficiency' was not observed. Nevertheless there was no evidence that lower intakes of protein in the group adversely influenced any measured variable. Indeed, both arm muscle circumference and a 'nutritional index' score calculated from albumin, triceps skinfolds and transferrin levels were inversely correlated with protein intakes, implying no deleterious impact of consuming protein at the lower end of the observed range.

Bunker *et al.* (1987) reported actual N balances for housebound elderly men and women (*n* 20, 70–86 years) with a mean protein intake of 0·67 g protein/kg per d and mostly in negative balance, and also for healthy men and women (*n* 24, 70–86 years) with a mean protein intake of 0·97 g protein/kg per d at zero balance overall. However, there was no indication that protein intake determined N balance. There was no correlation between protein intake and N balance in either group over a wide range of intakes (24–79 g protein/d in the housebound and 35–92 g protein/d in the healthy group). Furthermore, at the same intakes housebound subjects tended to be in negative balance whilst the healthy subjects were in positive balance. The immobility, or illness, or the lower energy intake of the housebound accounted for the negative N balance. Notwithstanding the limitations of such measurements, the findings do not support any effect of protein intake on N balance over a range of intakes as wide as those likely to be observed in a free-living population.

Thus, these two studies both point to free-living elderly individuals being able to adapt to protein intakes over a wide range, with no benefit in terms of either biochemical indicators or measured N balance from increased intakes. When assessed by specific N-balance studies, Millward & Roberts (1996) were unable to identify any convincing evidence for a revision of the FAO/WHO/UNU (1985) recommendations, and concluded that there appeared to be no change with age in the protein requirement per kg body weight. Indeed, they raised the possibility that the actual biological demand for protein and amino acids in the elderly is lower than that in younger adults.

Furthermore, as reviewed by Millward *et al.* (1997), this finding of no change with age is generally consistent with reports that the rate of whole-body protein turnover, a commonly-assumed determinant of the protein requirement, exhibits minimal change with age per unit fat-free mass. Our recent tracer studies, aimed at evaluating protein requirements and turnover in a systematic way, also support the FAO/WHO/UNU (1985) recommendations. We have reported [1–^{13}C]leucine-balance studies which allow measurement of metabolic demand (from post-absorptive leucine oxidation) and the efficiency of protein utilization from changes in leucine balance with feeding (Fereday *et al.* 1997). The apparent protein requirement is then calculated as metabolic demand/efficiency, an indication of protein needs and utilization during a standardized protocol at intakes similar to the habitual level. We applied this new approach in a study of healthy mobile elderly subjects and showed that because metabolic demands are reduced by about one-third, with no significant impairment in efficiency of protein utilization, apparent protein requirements also

appear to fall with age at similar rates for both men and women (see Fig. 2). These changes in the elderly reflect subtle changes in protein turnover, i.e. an improved restraint of proteolysis in the post-absorptive state, with little change with age in whole-body protein synthesis. Clearly, further studies of the requirements of those elderly subjects who are frail and immobile are needed using these new methods.

In summary, as far as optimum protein intakes for the elderly are concerned, it would appear that since protein requirements appear to fall rather than increase with age, and since there appears to be no benefit from increased protein intakes in relation to body composition or N balance, there is little justification for any revision of the current recommendations that no special additional allowance should be made above that of younger adults.

Optimum protein intakes for adults

The metabolic background

Although current adult requirement values are under review, and notwithstanding the continuing debate about N-balance studies (Millward, 1999*b*), it is difficult to identify new data likely to challenge the current values of 0·6 and 0·75 g/kg per d for the estimated average requirement and reference nutrient intake respectively. With diets throughout the developed world providing much higher intakes of both protein and indispensable amino acids than the reference nutrient intake (even for most vegetarians), inadequacy is now a much more limited issue, with the main current debate focusing on quality rather than quantity (see Millward, 1999*a*). It is appropriate to focus here on a better definition of the upper safe limit and the metabolic response to increasing intakes.

In fact, combined N-balance and stable-isotope amino acid tracer studies have afforded a much better insight into the adaptive response to varying dietary protein intake levels. This subject has been extensively reviewed recently
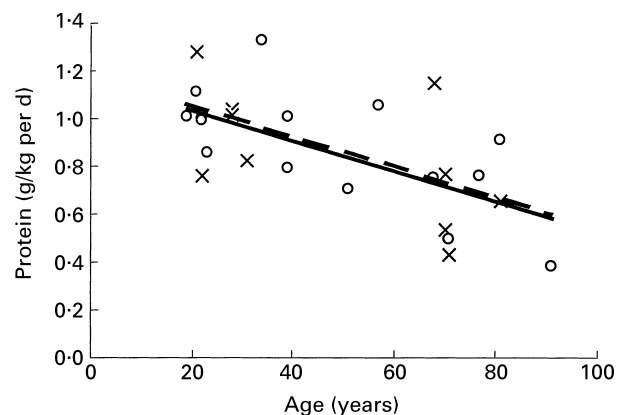

Fig. 2. Changes with age in the apparent protein requirements. Values were determined from [1-^{13}C]leucine-balance studies of metabolic demand and efficiency of utilization (for details, see Fereday *et al.* 1997; Millward *et al.* 1997). Linear regressions are shown for men (– –; $y = -0.0064x + 1.18$; r 0·45, n 15) and women (——; $y = -0.0063x + 1.16$; r 0·33, n 10). Both sexes are described by: $y = -0.0064x + 1.17$; r 0·39, n 25.

(Millward, 1999*a,b*), and only the salient points will be identified here.

The key advance is the identification of an adaptive component of the metabolic demand. Thus, in adults maintaining weight and in N equilibrium the metabolic demand for amino acids which determines the protein requirement includes three components: net protein synthesis (i.e. the repletion of post-absorptive losses and any growth); irreversible amino acid conversion into essential metabolites; oxidative catabolism of amino acids at a rate which varies with the habitual protein intake and which occurs continuously throughout the post-absorptive and post-prandial periods of the day. The latter component is distinct from oxidative catabolism due to any inefficiency of protein utilization or due to intakes which are acutely in excess of usual intakes, and changes in this adaptive metabolic demand occur only slowly (periods of weeks or months) in response to changes in intake. The consequence for N homeostasis of this demand is a diurnal cycle of fasting losses and fed-state gains of increasing amplitude with increasing habitual intake. Our studies in subjects consuming intakes up to 3·5 g protein/kg per d show no apparent limit to the amplitude of this diurnal cycle (Price *et al*. 1994). The mechanisms of these fed-state gains and fasting losses are mainly changes in rates of proteolysis (increases with fasting and decreases with feeding), with much smaller changes in protein synthesis. Most importantly, when average daily rates of protein turnover are calculated no marked changes are identified over a wide range of protein intakes (0·36–2·07 g/kg per d; Pacy *et al*. 1994). Thus, apart from exhibiting transient losses and repletion throughout each day, the turnover of the protein mass does not seem to be influenced by the level of intake. What appears to be influenced is the activity of the key enzymes involved in amino acid oxidation and N excretion, especially pathways relating to the indispensable amino acids.

Our work in growing animals indicated that dietary protein intakes in excess of those associated with maximum efficiency of protein utilization were associated with identifiable hormonal responses (insulin, thyroid hormones and insulin-like growth factor-1) and associated metabolic responses (increased rates of linear bone growth), resulting in the concept of an 'anabolic drive' of dietary protein exerted by protein intakes in excess of minimal needs (see Millward & Rivers, 1989). In contrast, our studies in adults over a wide range of protein intakes failed to identify any marked hormonal variations (see Pacy *et al*. 1994). While insulin levels increased with feeding, there were no dietary effects in either fasted or fed states. For insulin-like growth factor-1 no consistent feeding effect was observed, and the dietary influence was small and limited to a marginally significant increase in fasting levels from the lowest to highest level. Neither total nor free triiodothyronine levels changed with dietary protein intake, either in the fed or fasted state.

This finding would suggest to us that for the human organism adaptation to slow growth and weight stability over long periods has resulted in a relative metabolic insensitivity to protein intakes over a wide range, apart from those adaptive responses associated with the rapid disposal of dietary amino acids.

Definition of the adult protein requirement

Against this metabolic background it is clear that balance is likely to remain the main indicator of adequacy. A minimal requirement might be defined as the minimum intake which allows overall body protein or N balance, defined in terms of balance and the level of body N. As discussed elsewhere (Millward, 1995), it is likely that there is an upper limit of body protein; a function of height, frame size and most probably part of the genetic canalization. Maintaining this maximum body protein mass should be a nutritional objective, so that definition of minimum requirements would become the achievement of balance at a level of body protein judged to be the appropriate level for the subject's height and frame size.

On this basis, losses of body N and achievement of balance at a lower body N level would imply an inadequate intake. However, such losses of body N and achievement of balance at a lower body N level must be differentiated from any transient losses during an adaptation period which are gradually replaced, thus restoring appropriate body protein stores. Generally, this restoration stage has been ignored in most studies, probably because it takes so long. If changes in body composition, or at least in skeletal muscle mass, are excluded from any discussion of adaptation, then the discussion is simplified theoretically, and the so-called adapted lean individual with a reduced muscle mass compared with height and frame size is identified as malnourished. Experimentally, however, the problem becomes more difficult, since studies need to be long enough not only for balance to be achieved at the lowered level but also for repletion of losses induced during the adaptation to occur, and this process will be most difficult to measure.

Although there have been few attempts to investigate long-term adaptation to low intakes (see FAO/WHO/UNU, 1985), in theory such a minimal protein requirement may be identifiable with sufficient time allowed for adaptation. In practice the current value of 0·6 g/kg per d derives mainly from short-term balances, so that it is more likely to be an overestimate rather than underestimate. In any case, the true minimal requirement is likely to be so much lower than the amounts provided by natural diets (which are providing sufficient energy and other nutrients) that its magnitude becomes to some extent an issue of scientific curiosity only. The minimum requirement for balance becomes of less importance than the functional consequences of particular levels of protein in the diet. Thus, for the adult, in the absence of sensitive metabolic indicators of adequacy, the issue of an optimal requirement which not only allows balance but also supports optimal body function becomes the crucial issue.

Cost of intakes above the minimal requirement

Renal function.   In the Department of Health (1991) report it was stated that whilst in no case is there conclusive evidence for harmful effects of excessive intakes of dietary protein in healthy people, there is firm evidence that excessive dietary protein contributes to deterioration of renal function in patients with renal disease by increasing intraglomerular pressure and glomerular filtration rate

(GFR) (Brenner *et al.* 1982; Wiseman *et al.* 1987; Rudman, 1988). It also highlighted the fact that since dietary protein increases the GFR in normal subjects, it had been suggested (for example, see Brenner *et al.* 1982) that protein intake could be a risk factor in the age-related decline in renal function. Whilst the issue of restriction of protein intake is clear in relation to renal disease (Mitch & Maroni, 1998), for healthy subjects the view of Brenner *et al.* (1982) has by no means been confirmed, and the debate continues as to the extent of any adverse influences of high protein intakes for normal renal function. Indeed, several recent studies challenge this view. First, Kimmel *et al.* (1996) pose the question of whether renal function does inevitably decline with age. They report that in a carefully characterized sub-population of subjects there is no decrease in GFR over time, and these authors view the GFR as a physiological variable with protein intake an important determinant. Thus, low levels of GFR when they are observed in, for example, elderly subjects may not indicate any renal disease, but may be a simple reflection of a lower protein intake. This view is consistent with the studies of Brandle *et al.* (1996), who report on the relationship between protein intake, GFR and albumin excretion in healthy volunteers with a wide range of protein intakes (body-builders with and without protein supplements and vegetarians). Whilst they clearly show that chronic oral protein intake is a key controller of the GFR, no relationship between protein intake and albumin excretion was observed. It is clear that such studies do not negate the possibility of risk to renal function from high protein intakes, but they do raise the possibility that the supposed problem might be more apparent than real.

Indeed, Walser (1992) has suggested, contrary to the view of Brenner *et al.* (1982), that the decline in renal function with ageing and the progressive increase in sclerotic glomeruli in ageing kidneys might be attenuated by restricting dietary protein; in fact, the opposite is more likely to be the case. Walser (1992) argues that: (1) symptomatic renal failure does not result from the physiological decrease in GFR with age because symptoms do not occur until GFR is one-quarter of normal or less, a far greater decrease than that caused by ageing; (2) protein restriction lowers GFR instead of raising it, and the decrease in spontaneous protein intake with age is the major cause of the decline in GFR with age; (3) there is evidence that the decrease in creatinine clearance with age is independent of protein intake.

Walser (1992) argues that there is in fact no evidence that high protein intake can initiate renal disease (other than nephrolithiasis). Even in kidney donors or in subjects who have only one functioning kidney owing to congenital malformation there is no clear rationale for restricting protein intake. While initial experiments in rats suggested that reduction in hyperfiltration caused by reduced protein intake could protect against progressive kidney damage, it was later shown that reduced energy intake secondary to the lower proportion of protein in the diet was the explanation for these results (Tapp *et al.* 1989).

Walser (1992) concludes that only in subjects who are likely to develop kidney failure owing to diabetes, hypertension, or polycystic kidney disease is protein restriction prudent, even in the absence of compelling evidence.

Bone health. The Department of Health (1991) report briefly referred to evidence indicating that excessive dietary protein may contribute to demineralization of bone (Garn & Kangas, 1988), pointing out that the relationship was by no means a simple one (Orwoll *et al.* 1987), and that in any case any such effect may be minimized by the increases in dietary P which can accompany increased dietary protein (Hegsted *et al.* 1981). Nevertheless, reports suggesting that osteoporosis is less marked in vegetarians compared with omnivores (for example, see Ellis *et al.* 1972) are interpreted as a reflection of their lower protein intake. The interaction between intakes of Ca, protein and P in relation to Ca balance have been reviewed by Heaney (1993).

The calciuretic effect is usually considered to be due to an increased GFR associated with increased amino acid concentrations, resulting in an increased filtered Ca load, coupled with a decreased tubular Ca re-absorption related to the mild acidosis associated with sulfate excretion from S-containing amino acids. The higher protein intake of 70 g/d in omnivores compared with 55 g/d in vegetarians increases acid excretion from 35 mmol/d to 49 mmol/d (Robertson & Maughen, 1992). The acidosis effect appears to be counteracted in part by dietary P (intakes often correlated with animal protein and meat), which increases the renal tubular re-absorption of Ca and reduces urinary excretion of Ca. Indeed, P has been shown to decrease urinary Ca regardless of Ca intake.

The issue has never been straightforward and is by no means clear today, especially in relation to the Ca–P interaction and the extent to which the mild protein-induced acidosis influences Ca balance. Thus, Orwoll *et al.* (1987) reported that whilst in young men and post-menopausal women dietary protein can induce a hypercalciuria resulting in negative Ca balance, this effect is blunted if the protein is part of a mixed diet and is not observed at moderately increased intakes of protein, suggesting an effect limited to very high protein intakes. Furthermore, with bone mineral content in ageing normal men as the outcome, these workers reported no direct relationship between protein intake and the fall in bone mineral content with age.

More recently, several reports have appeared which raise doubts about whether the protein-induced calciuresis can be simply related to overall bone health. Shapes *et al.* (1995) reported an intervention study with both protein and Ca in healthy subjects, focusing on bone resorption as assessed by urinary pyridinium cross-link excretion. They showed that whilst changes in Ca from 429 mg to 1589 mg lowered cross-links by 33 %, increases in protein intake from 0·49 g/kg per d to 2·71 g/kg per d had no influence on resorption assessed in this way. Cooper *et al.* (1996) reported on protein intake and bone mass in pre- and post-menopausal women in the northern USA, showing a positive association in premenopausal women but no relationship in post-menopausal women. Finally, the results of a careful Ca-balance protein-supplementation study in young and elderly subjects point to benefit rather than any adverse influence (Pannemans *et al.* 1997). By assessing both urinary and faecal losses, Ca excretion, apparent absorption and overall balance were measured in subjects fed on diets with P : E values of either 12 or 21 for 3 weeks in a randomized crossover design. Ca excretion in faeces

(% intake) was lower during the higher protein intake, whilst calciuresis during the higher protein intake was only observed in the young subjects. The net effect of these responses was no influence on Ca balance in young adults, but improved balance in the elderly.

The findings of Pannemans *et al.* (1997) are clearly important if they can be reproduced, since to date it is the only study indicating benefit of increased protein intakes for the elderly, and as reviewed by these authors the numerous studies in young and middle-aged adults over the last two decades present an inconsistent set of data for Ca balance.

It would appear that much more direct intervention work is needed in this area, focusing on direct indicators of bone mineral balance, metabolism and overall health, and that there is little further to gain by simple studies of urinary Ca losses and diet. Until this problem is resolved, it is clearly premature to use bone health as an indicator of optimal protein intakes.

Benefit of intakes above the minimal requirement

As far as benefits are concerned the importance of dietary protein for the maintenance of the immune system has been suggested, with dietary protein and specific amino acids influencing the response to infection through several mechanisms (gut-barrier function through provision of threonine and cysteine, general immunocompetence, GSH synthesis from glutamate (glutamine), glycine and cysteine, and specific metabolic roles of glutamine, arginine and possibly taurine; see Reeds & Becket, 1996). However, no quantitative relationship between intakes of any of these individual amino acids and function exists, or is likely to be identified in the near future.

Blood pressure and stroke. In the Department of Health (1991) report it was noted that populations consuming vegetarian diets containing on average lower protein intakes than other groups exhibit lower blood pressure, which was based on the report of Prescott *et al.* (1988). However, since 1991 data have accumulated suggesting the opposite relationship, i.e. that stroke mortality is associated inversely with protein (as well as fat) intake (Klag & Whelton, 1993), and there is considerable evidence relating protein intakes inversely with blood pressure. Thus, the effect on blood pressure has now become one of the few examples of apparent benefit resulting from increasing protein intakes. Thus, Obarzanek *et al.* (1996) list nine cross-sectional surveys of American and British adults showing that increased protein intake lowers blood pressure; one American study showing the same relationship with vegetable protein and three studies in China and Japan showing the same relationship with animal protein. Protein intakes varied inversely with blood pressure in the Multiple Risk Factor Intervention Trial (Stamler *et al.* 1996*a*). The largest single study (INTERSALT; *n* 10 020; Stamler *et al.* 1996*b*), showed that an increase in protein intakes from 55–100 g/d lowers diastolic blood pressure by 2·9 mmHg. So far the relationship remains suggestive of a direct benefit from increasing protein intakes, since intervention studies have mostly found no significant effects of protein on blood pressure (Obarzanek *et al.* 1996) and, apart from the work on low-protein diets in pregnancy, few animal studies have

specifically examined the effects of increased dietary protein on blood pressure. Also, the issue of the type of dietary protein is not entirely clear.

As far as the mechanism is concerned Obarzanek *et al.* (1996) review several hypotheses which have been advanced, the main ones being stimulatory effects of increased amino acids on renal plasma flow and Na excretion, influences on catecholamine metabolism, and influences on NO and vascular tone through the effects of supply of arginine on NO synthesis and cysteine on NO turnover metabolism.

Assuming the epidemiological data prove to reflect a causal relationship, then the magnitude of the influence would indicate public health implications. An increase of 37 g/d would result in a fall in systolic and diastolic blood pressure of 3·0 and 2·5 mmHg respectively.

Athletes. The protein intakes of athletes is particularly important in the present context, not only in relation to any benefit accruing from increased intakes, but also because they represent a group with high levels of energy expenditure and intake. Thus, their protein intakes are likely to be high anyway, and definition of a safe upper limit will be of particular relevance.

Benefit of increased intakes for athletes is widely believed and practised, as evident by the range of protein supplements available and used in gyms and health centres around the world. The promotional material for such supplements is supported by selected scientific litererature. Thus, Lemon (1996) argues that the recommended dietary allowance for those who engage in regular endurance exercise should be about 1·2–1·4 g protein/kg body mass per d (150–175 % of the current recommended dietary allowance) and 1·7–1·8 g protein/kg body mass per d (212–225 % of the current recommended dietary allowance) for strength exercisers. As previously discussed (Millward *et al.* 1994), much of the information indicating apparent increased protein requirements of athletes is based on arguably misleading N-balance studies, with very few data supporting benefit in terms of performance. A case in point is a study of protein requirements and muscle mass and strength changes during intensive training in novice bodybuilders (Lemon *et al.* 1992). In this randomized double-blind crossover study intensive bodybuilders were supplemented with either protein to provide 2·62 g/kg per d or carbohydrate to provide 1·35 g/kg per d for 1 month during intensive weight training, with 3 d N-balances after 3·5 weeks on each treatment. Measurements were reported of strength (voluntary and electrically evoked) and muscle mass (density, creatinine excretion, muscle area by computer axial tomography scan, and biceps N content). As is usual in subjects on high-protein diets, a marked positive balance was achieved with the high intake (9 g N/d) with a negative balance for the control (−3·4 g N/d). The excessive positive balance is consistent with many previous reports of responses to high protein intakes (Hegsted, 1976; Oddoye & Margen, 1979). A positive balance of 9 g N/d is unlikely to be tissue protein, since if it was it would be measurable (a gain of 2 kg/week or 7 kg fat-free mass over the study compared with a loss on the control intake of 0·7 kg/week or 2·6 kg fat free-mass over the study, i.e. an overall difference between treatments of 9·6 kg fat-free mass). No such difference was observed, and

the attempt to define a requirement from such balance data (reported as 1·45 g/kg per d), whilst understandable, is in fact unwise. Since no difference in muscle mass or performance was reported between the two dietary groups, it should be this lack of difference which is of most concern to bodybuilders. In other words, the significance of either the positive or negative balances cannot be interpreted, given the absence of any differences in the carefully measured body composition and performance indicators.

In a previous review of physical activity, protein metabolism and protein requirements (Millward *et al.* 1994) it was concluded that in untrained subjects bouts of unaccustomed exercise do provoke N losses in variable amounts according to their type and intensity and the immediately-previous nutritional state. However, for the appropriately-trained individual with adequate energy supply, N losses associated with physical activity may be minimal or even less than those in sedentary individuals. On this basis the relationship between protein needs for overall balance and physical activity was described by a U-shaped curve, with needs increasing in inactive subjects and with intense activity, but the extent of any increase with activity reduced by both training and energy provision and increased by increasing dietary protein.

The key question is whether there is risk for athletes associated with high protein intakes. Three potential problems can be defined. The first problem relates to the impact of exercise on leucine oxidation and amino acid catabolism in general. It has been shown that the exercise-induced increases in leucine oxidation are higher when the habitual protein intakes increase (Millward *et al.* 1994). Thus, high protein intakes may increase requirements for athletes. Second, since the adaptation to a lower intake involves considerable loss of body N (Quevedo *et al.* 1994), athletes on protein supplements who relax such diets (e.g. on nontraining days) are likely to lose body N, negating any gain which might have occurred. Third, according to the findings of Chittendon (1907), high-protein diets may be detrimental to performance by increasing perceived fatigue. Chittendon (1907) took a group of elite University of Yale athletes and persuaded them to reduce their protein intake by 50 % over 5 months, mainly by switching to a vegetarian diet of about 0·75 g protein/d. Extensive measures of strength were made. Over the 5 months their strength increased on average by 35 %, coupled with a fall in perceived fatigue. Chittendon (1907) defined an optimal requirement of about 0·75 g/kg, mainly from plant-protein sources. This value is the current UK dietary reference value, and it is significantly less than the average UK intake. Those researchers aware of the work of Chittendon (1907) often dismiss it as 'unpublishable today' (no measures of dietary compliance, poor measures of strength). However, apart from the absence of a control group, the work in my view is convincing and does stand up to scrutiny. Whilst athletes on protein intakes as low as those of the athletes studied by Chittendon (1907) are rare given the culture of the average gym, in a group of twentysix bodybuilders of both sexes studied by us, with protein intakes from food alone averaging 1·93 (SD 0·46) g/kg per d, individual values ranged from 0·63 g/kg (a lacto-ovo vegetarian) to 3·05 g/kg with diets of P : E values ranging from 8·2 to 28. As far as outcomes were concerned, there was no discernible impact of these different intakes, and the vegetarian bodybuilder had won as many awards as others in the group.

Definition of the safe upper limit

As to the wider issue of the practicality of defining a safe upper limit, it is in fact difficult for athletes or any other individuals with high levels of energy expenditure to avoid increasing protein intakes. The total energy expenditure (measured using the doubly-labelled-water method) of a series of male bodybuilders at 79·6 kg was 15·4 MJ (3700 kcal)/d (i.e. a physical activity level of 1·96) and their measured dietary P : E was 21·5 (2·5 g/kg per d; Quevedo *et al.* 1992). It is interesting to calculate what range of intakes could be consumed by such a group assuming usual UK diets. Consuming food of average UK composition (P : E 14·3; Jackson & Margetts, 1993) would provide 1·66 g/kg, or for a meat-free lacto-ovo diet (P : E 12·6) 1·46 g/kg. Assuming their diet was the average for the UK (P : E 14·3) and that their excess energy expenditure over average sedentary adults (about 3·1 MJ (740 kcal)/d) was derived only from carbohydrate sources of rice, potatoes or bread, their protein intakes would range from 1·35 g/kg to 1·54 g/kg on a base diet if meat-free lacto-ovo vegetarians, or from 1·5 g/kg to 1·7 g/kg if omnivores.

Extending the argument, Fig. 3 shows the relationship between energy expenditure and protein intakes for subjects consuming diets of increasing P : E values, ranging from very-low rice-based vegetarian diets in India (Pellett, 1996), to UK non-meat and omnivore diets (Jackson & Margetts, 1993) to the diets of our bodybuilders (Quevedo *et al.* 1992). It is clear that if 1·5 g/kg per d was a safe upper limit, then whilst individuals on the vegetarian diets consumed in India would not exceed this level up to a physical activity level of
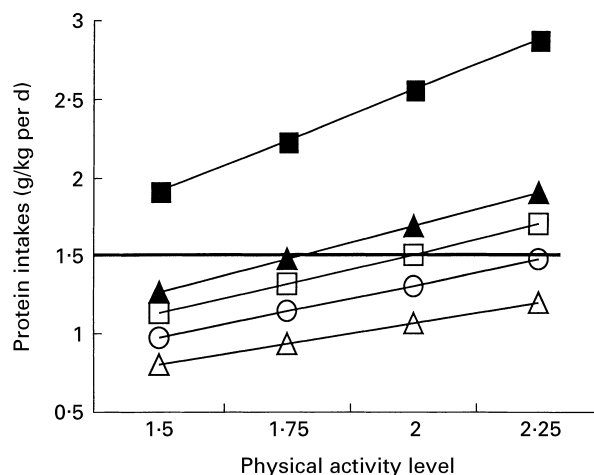

Fig. 3. Relationship between protein intake and energy expenditure on various diets. Values were calculated for adults with a body weight of 79·6 kg and BMR of 7·9 MJ (1893 kcal)/d. Protein : energy values for the habitual diets were: India (○) 11, West Bengal (△) 9 (Pellett, 1996); UK vegetarians (□) 12·7, UK omnivores (▲) 14·2 (Jackson & Margetts, 1993); bodybuilders (■) 22 (Quevedo *et al.* 1992; MR Quevedo, PJ Pacy and DJ Millward, unpublished results).

2·25, UK lacto-vegetarians would exceed this level above a physical activity level of 2, and so would UK omnivores above a physical activity level of 1·75. Thus, it is difficult to see how athletes and physically-active subjects can eat less than about twice the current recommended dietary allowance (1·5 g/kg per d) without adopting a high-fat diet (clearly inappropriate) or deliberately avoiding any protein-dense foods, as with the athletes of Chittenden (1907). If 1·5 g/kg per d is to be defined as a safe upper limit then active subjects would have to change their diet to fewer protein-rich foods, i.e. more carbohydrate staples.

Conclusions

Defining optimal intakes of protein in the human diet requires indicators of adequacy which are unequivocal and quantifiable, and it is clear that progress in identifying such indicators is painfully slow.

As far as issues to do with definition of requirements are concerned, for those groups likely to be thought of as having special needs and being at risk of deficiency, only in the case of infants and children does there appear to be any case for any revision in current guidelines, and this change is to lower values which would appear to be safe when consumed as formula. Thus, in pregnancy, while some workers have posed the question of whether deficiency is a problem, implying that the requirement should be increased, in fact there is little evidence of any increased need and some evidence that increased intakes would pose a risk. For the elderly there appears to be no evidence that their requirement is higher than that of younger adults and no evidence of benefit from increased intakes. For adults, while we now know much more about metabolic adaptation to varying intakes and can better explain the metabolic demand for and utilization of protein, there would appear to be little evidence warranting a change in current recommendations. The question of whether athletes need much more protein will undoubtedly remain controversial, given the commercial interests in protein and amino acid supplements, but clear evidence of benefit in terms of performance is hard to find. As far as issues to do with definition of safe upper limits are concerned, it would appear that the somewhat arbitrary value of twice the reference nutrient intake suggested by the Department of Health (1991) requires careful reconsideration, because unless individuals with high rates of energy expenditure and intakes markedly change their patterns of food intake, 1·5 g/kg per d is easily exceeded and there is little clear evidence that this level would be associated with risk. Indeed, if the apparent benefit of increased protein intakes on blood pressure and stroke mortality proves to be a true causal relationship, there would be a strong case for revising the use of a safe upper limit. The risks of high protein intakes on renal function and bone health continue to be raised, but the scientific case for these risks is now weaker than it was in 1991. One potential area which is unexplored is the dietary S-amino acid intake in relation to homocysteine levels and associated morbidity. While there is no reason to expect a simple relationship between methionine intake and plasma homocysteine levels, it remains to be seen if this assumption is correct.

References

Barker DJ (1998) *In utero* programming of chronic disease. *Clinical Science* **95**, 115–128.

Beaton GH & Cheery A (1988) Protein requirements of infants: a re-examination of concepts and approaches. *American Journal of Clinical Nutrition* **48**, 1403–1412.

Brandle E, Sieberth HG & Hautmann RE (1996) Effect of chronic dietary protein intake on the renal function in healthy subjects. *European Journal of Clinical Nutrition* **50**, 734–740.

Brenner BM, Meyer TW & Hostetter TH (1982) Dietary protein intake and the progressive nature of kidney disease: the role of hemodynamically mediated glomerular injury in the pathogenesis of progressive glomerular sclerosis in aging, renal ablation, and intrinsic renal disease. *New England Journal of Medicine* **307**, 652–659.

Bunker VW, Lawson MS, Stanfield ME & Clayton BE (1987) Nitrogen balance studies in apparently healthy elderly people and those who are housebound. *British Journal of Nutrition* **47**, 211–221.

Campbell DM, Hall MH, Barker DJ, Cross J, Shiell AW & Godfrey KM (1996) Diet in pregnancy and the offspring's blood pressure 40 years later. *British Journal of Obstetrics and Gynaecology* **103**, 273–280.

Chittenden RH (1907) *The Nutrition of Man*. London: Heinemann.

Cohen RJ, Brown KH, Canahuati J, Rivera LL & Dewey KG (1995) Determinants of growth from birth to 12 months among breast-fed Honduran infants in relation to age of introduction of complementary foods. *Pediatrics* **96**, 504–510.

Cooper C, Atkinson EJ, Hensrud DD, Wahner HW, O'Fallon WM, Riggs BL & Melton LJ (1996) Dietary protein intake and bone mass in women. *Calcified Tissue International* **58**, 320–325.

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 (1997) Energy and protein requirements during lactation. *Annual Reviews of Nutrition* **17**, 19–36.

Dewey KG, Beaton G, Fjeld C, Lonnerdal B & Reeds P (1996a) Protein requirements of infants and children. *European Journal of Clinical Nutrition* **50**, Suppl. 1, S119–S147.

Dewey KG, Cohen RJ, Rivera LL, Canahuati J & Brown KH (1996b) Do exclusively breast-fed infants require extra protein? *Pediatric Research* **39**, 303–307.

Dewey KG, Heinig MJ, Nommsen LA, Peerson JM & Lonnerdal B (1992) Growth of breast-fed and formula-fed infants from 0 to 18 months: the DARLING Study. *Pediatrics* **89**, 1035–1041.

Dewey KG, Heinig MJ, Nommsen LA, Peerson JM & Lonnerdal B (1993) Breast-fed infants are leaner than formula-fed infants at 1 y of age: the DARLING study. *American Journal of Clinical Nutrition* **57**, 140–145.

Dewey KG, Heinig MJ & Nommsen Rivers LA (1995) Differences in morbidity between breast-fed and formula-fed infants. *Journal of Pediatrics* **126**, 696–702.

Ellis FR, Holesh FRC & Ellis JW (1972) Incidence of osteoporosis in vegetarians and omnivores. *American Journal of Clinical Nutrition* **25**, 555–558.

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.

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.

Fomon SJ (1991) Requirements and recommended dietary intakes of protein during infancy. *Pediatric Research* **30**, 391–395.

Fomon SJ, Ziegler EE, Nelson SE & Franz JA (1995) What is the safe protein-energy ratio for infant formulas? *American Journal of Clinical Nutrition* **62**, 358–363.

Garn SM & Kangas J (1988) Protein intake, bone mass and bone loss. In *Osteoporosis: Recent Advances in Pathogenesis and Treatment*, pp. 212–230 [HF De Luca, editor]. Baltimore, MD: University Park Press.

Godfrey KM, Barker DJ, Robinson S & Osmond C (1997) Maternal birthweight and diet in pregnancy in relation to the infant's thinness at birth. *British Journal of Obstetrics and Gynaecology* **104**, 663–667.

Godfrey KM, Forrester T, Barker DJ, Jackson AA, Landman JP, Hall JS, Cox V & Osmond C (1994) Maternal nutritional status in pregnancy and blood pressure in childhood. *British Journal of Obstetrics and Gynaecology* **101**, 398–403.

Godfrey K, Robinson S, Barker DJ, Osmond C & Cox V (1996) Maternal nutrition in early and late pregnancy in relation to placental and fetal growth. *British Medical Journal* **312**, 410–414.

Heaney RP (1993) Protein intake and the calcium economy. *Journal of the American Dietetic Association* **93**, 1259–1260.

Hegsted DM (1976) Balance studies. *Journal of Nutrition* **106**, 307–311.

Hegsted DM, Schuette SA, Zemel MB & Linkswiler HM (1981) Urinary calcium and calcium balance in young men as affected by the level of protein and phosphorus intake. *Journal of Nutrition* **111**, 553–562.

Heinig MJ, Nommsen LA, Peerson JM, Lonnerdal B & Dewey KG (1993) Energy and protein intakes of breast-fed and formula-fed infants during the first year of life and their association with growth velocity: the DARLING Study. *American Journal of Clinical Nutrition* **58**, 152–161.

Jackson AA & Margetts BM (1993) Protein intakes in the adult population of the UK. *International Journal of Food Sciences and Nutrition* **44**, 95–104.

Kimmel PL, Lew SQ & Bosch JP (1996) Nutrition, ageing and GFR: is age-associated decline inevitable? *Nephrology Dialysis and Transplantation* **11**, Suppl. 9, 85–88.

Klag MJ & Whelton PK (1993) The decline in stroke mortality. An epidemiologic perspective. *Annals of Epidemiology* **3**, 571–575.

Langley SC & Jackson AA (1994) Increased systolic blood pressure in adult rats induced by fetal exposure to maternal low protein diets. *Clinical Science* **86**, 217–222.

Lemon PW (1996) Is increased dietary protein necessary or beneficial for individuals with a physically active lifestyle? *Nutrition Reviews* **54**, S169–S175.

Lemon PW, Tarnopolsky MA, Macdougall JD & Atkinson SA (1992) Protein requirements and muscle mass/strength changes during intensive training in novice bodybuilders. *Journal of Applied Physiology* **73**, 767–775.

Millward DJ (1995) A protein-stat mechanism for the regulation of growth and maintenance of the lean-body mass. *Nutrition Research Reviews* **8**, 93–120.

Millward DJ (1999*a*) 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 (1999*b*) Metabolic demands for amino acids and the human dietary requirement: Millward and Rivers (1988) revisited. *Journal of Nutrition* (In the Press).

Millward DJ, Bowtell JL, Pacy P & Rennie MJ (1994) Physical activity, protein metabolism and protein requirements. *Proceedings of the Nutrition Society* **53**, 223–240.

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 & Rivers JP (1989) The need for indispensable amino acids: the concept of the anabolic drive. *Diabetes Metabolism Reviews* **5**, 191–211.

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

Mitch WE & Maroni BJ (1998) Nutritional considerations and the indications for dialysis. *American Journal of Kidney Diseases* **31**, 185–189.

Munro HN, McGandy RB, Hartz SC, Russell RM, Jacob RA & Otradovec MA (1987) Protein nutriture of a group of free-living elderly. *American Journal of Clinical Nutrition* **46**, 586–592.

Obarzanek E, Velletri PA & Cutler JA (1996) Dietary protein and blood pressure. *Journal of the American Medical Association* **275**, 1598–1603.

Oddoye LA & Margen S (1979) Nitrogen balance studies in humans: long-term effect of high nitrogen intake on nitrogen accretion. *Journal of Nutrition* **109**, 363–377.

Orwoll ES, Weigel RM, Oviatt SK, Meier DE & McClung MR (1987) Serum protein concentrations and bone mineral content in aging normal men. *American Journal of Clinical Nutrition* **46**, 614–621.

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.

Pannemans DLE, Schaafsma G & Westerterp KR (1997) Calcium excretion, apparent calcium absorption and calcium balance in young and elderly subjects: influence of protein intake. *British Journal of Nutrition* **77**, 721–729.

Pellett PL (1996) World essential amino acid supply with special attention to South-East Asia. *Food and Nutrition Bulletin* **17**, 204–234.

Prescott SL, Jenner DA, Beilin LJ, Margetts BM & Vandongen R (1988) A randomized controlled trial of the effect on blood pressure of dietary non-meat protein versus meat protein in normotensive omnivores. *Clinical Science* **74**, 665–672.

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, Cox M, Coward WA, Jones D, Pacy P, Smeaton I, Thorpe D & Millward DJ (1992) Energy intake and expenditure in bodybuilders. *Proceedings of the Nutrition Society* **50**, 238A.

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.

Reeds PJ & Becket PR (1996) Protein and amino acids. In *Present Knowledge in Nutrition*, 7th ed., pp. 67–86 [EE Ziegler and LJ Filer, editors]. Washington, DC: ILSI Press.

Robertson JD & Maughen RJ (1992) Acid–base status of premenopausal vegetarian and omnivorous women. *Proceedings of the Nutrition Society* **51**, 32A.

Rudman D (1988) Kidney senescence; a model for aging. *Nutrition Reviews* **46**, 209–214.

Rush D (1989) Effects of changes in maternal energy and protein intake during pregnancy, with special reference to fetal growth. In *Fetal Growth*, pp. 98–105 [F Sharp, RB Fraser and RDG Milner, editors]. London: Springer Verlag.

Shapses SA, Robins SP, Schwartz EI & Chowdhury H (1995) Short-term changes in calcium but not protein intake alter the rate of bone resorption in healthy subjects as assessed by urinary pyridinium cross-link excretion. *Journal of Nutrition* **125**, 2814–2821.

Stamler J, Caggiula A, Grandits GA, Kjelsberg M & Cutler JA (1996*a*) Relationship to blood pressure of combinations of dietary macronutrients. Findings of the Multiple Risk Factor Intervention Trial (MRFIT). *Circulation* **94**, 2417–2423.

Stamler J, Elliott P, Kesteloot H, Nichols R, Claeys G, Dyer AR & Stamler R (1996b) Inverse relation of dietary protein markers with blood pressure. Findings for 10,020 men and women in the INTERSALT Study. INTERSALT Cooperative Research Group. INTERnational study of SALT and blood pressure. *Circulation* **94**, 1629–1634.

Tapp DC, Kobayashi S, Femandes G & Venkatachalam MA (1989) Calorie, not protein restriction prevents renal injury in the remnant kidney after nephrectomy in the rat. *Seminars in Nephrology* **9**, 43–353.

Walser M (1992) The relationship of dietary protein to kidney disease. In *Dietary Proteins in Health and Disease*, pp. 168–178 [GU Liepa, editor]. Washington, DC: American Oil Chemists Society.

Wiseman MJ, Hunt R, Goodwin A, Gross JL, Keen H & Viberti GC (1987) Dietary composition and renal function in healthy subjects. *Nephron* **46**, 37–42.