Physical activity, protein metabolism and protein requirements

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The extent to which physical activity influences protein requirements is a difficult question to answer on two counts. First, physical activity results in a complex and presently poorly understood set of physiological and metabolic responses which are variable according to the intensity, duration and qualitative nature of work performed (Rennie *et al.* 1994). Second, protein requirements for the normal individual are difficult to define and subject to controversy (Millward *et al.* 1989). It will not be surprising to find, therefore, that there is no consensus as to whether protein requirements are influenced by physical activity. Our objective here is to present an overview of the metabolic impact of exercise on amino acid and N metabolism and the consequences of that impact on dietary protein needs from the perspective of our own view of the metabolic basis of protein requirements.

METABOLIC BASIS OF PROTEIN REQUIREMENTS

For protein, as with most nutrients, it is useful to consider the nutritional requirement to consist of two components, intrinsic nutritional demands, and needs which result from the regulatory response to the environment (see Millward, 1993). Nutritional demands for protein include growth and replacement of obligatory N losses (ONL). The regulatory response of protein metabolism to the environment includes losses of amino acids and N due to food intake and other influences acting on regulatory processes associated with protein, amino acid and N homeostasis. This latter component is large since current estimates of protein requirement at all ages are such that nutritional demands (growth + ONL) are never much more than 50% of the total requirement, even less in the case of children.

A simple metabolic scheme describing this view of protein metabolism is shown in Fig. 1. It includes protein turnover, growth and two sorts of N loss, obligatory (L_o) and regulatory (L_r).

Growth and L_o are intrinsic, i.e. they reflect identifiable metabolic processes which are most likely to be fixed, and they constitute nutritional demands. Thus, physical activity would involve an increase in this component to the extent that there is growth of muscle and any other lean tissue, and any associated obligatory metabolic consumption of amino acids which could be specifically and quantifiably related to the activity.

The oxidative losses (L_r), represent the regulatory response, i.e. extra losses which reflect the metabolic cost of maintaining homeostasis in response to external influences. The main external influence is the habitual protein intake which sets the rate of N
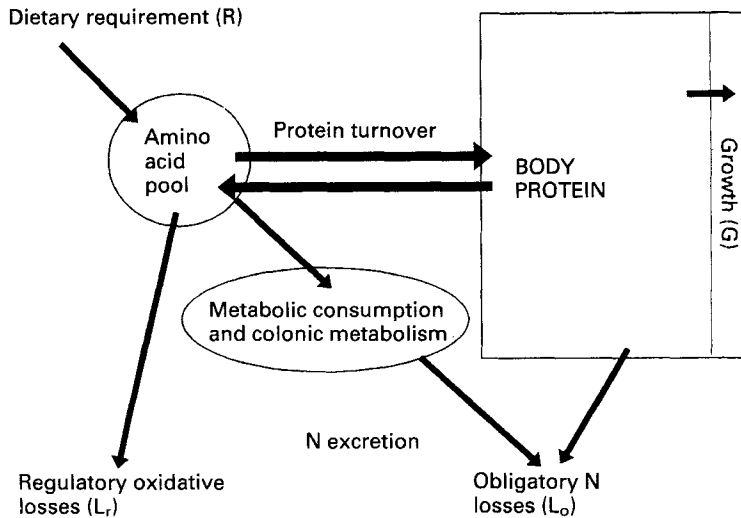
Fig. 1. A metabolic framework for amino acid requirements (see Millward & Rivers, 1988). The protein requirement (R) will be the sum of deposition (G) and losses (regulatory (L_r) and obligatory (L_o)): i.e. $R = G + L_r + L_o$. Since L_r is variable, especially with protein intake, R can only be defined in terms of minimum (R_{min}) and optimal ($R_{optimal}$) values: i.e. $R_{min} = G + L_{rmin} + L_o$, the lowest intake allowing balance after complete adaptation, and $R_{optimal} = G + L_{roptimal} + L_o$, the intake allowing balance and optimal performance after complete adaptation.

excretion (Price *et al.* 1994) with the capacity for adaptation to a wide range of protein intakes (Food and Agriculture Organization/World Health Organization/United Nations University, 1985). Any losses associated with physical activity that are variable and adaptive, with training and previous nutrition for example, would fall in this category. With habitual protein intake a major controlling influence on this category of losses, and especially with the possibility of interaction between protein intake and exercise on such losses, a dilemma arises. A judgement must be made as to whether a particular protein intake which satisfies obligatory metabolic needs is satisfactory, i.e. whether such intakes are optimal requirements. This can only be judged in terms of metabolic or functional tests of adequacy, which may be only distantly related to achievement of N balance. Millward & Rivers (1988) defined such influences as the anabolic drive, a tangible phenomenon in growing animals, but as yet uncharacterized in humans. Any beneficial influence of dietary protein level on physical performance would allow definition of an optimal requirement.

    Whilst definition of the influence of physical activity on protein requirements in terms of nutritional demands, regulatory responses and performance is an important objective, few studies have been designed from such a perspective and the current knowledge base is consequently inadequate for such an analysis with any certainty. Nevertheless, it is constructive to examine the literature in an attempt to separate fixed metabolic demands for protein from variable regulatory responses, and in what follows this will be done where possible.
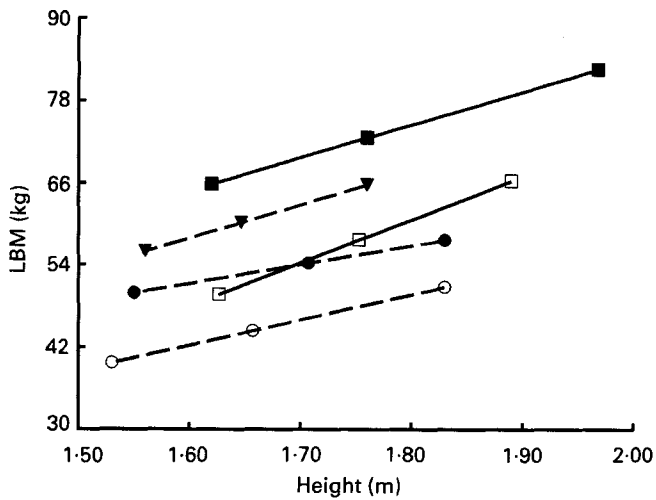
Fig. 2. Lean body mass (LBM) and height of male (□) and female (○) normal adults, male (■) and female (●) athletes and obese women (▼). The lines are the regressions drawn between minimum, mean and maximum values determined by densitometry (P. J. Pacy, M. R. Quevedo and D. J. Millward, unpublished results).

PHYSICAL ACTIVITY AND GROWTH OF LEAN BODY MASS (LBM)

Forbes (1985) reviewed the evidence relating LBM to physical activity, emphasizing the influences of height, sex, obesity and ethnic origin (LBM of Asians<caucasians<blacks). Calculation of any influence of physical activity on LBM needs to consider these variables. Our own findings are derived from body builders (male and female), elite rowers (male and female), none of whom were taking steroids, and obese women as well as normal controls of both sexes. These values are shown in Fig. 2 with the regressions of LBM v. height plotted between the minimum and maximum heights and with group mean values shown. The influence of sex, height and obesity are clearly apparent. Calculations made on the basis of similar height (1·7 m) indicate that excess LBM of male and female athletes were 15·5 and 8·1 kg respectively and that of obese women was 16·8 kg. Calculation of equivalent daily accretion rates which would result in such LBM expansion requires the time-course of the gain to be known, which we have not documented. For both male and female athletes, their training and competition had been proceeding for several years (obesity had been classified as such for >5 years). Assuming the gain in LBM was made over 3 years (a likely minimum) then the accretion rate is equivalent to a daily rate of 20–30 mg protein/kg per d for both groups of athletes, a trivial amount equal to 3–4% of the dietary reference value (DRV; Department of Health, 1991). In any case Forbes (1985) argued that athletes may simply be better endowed with skeletal muscle at the outset, since in longitudinal studies he was unable to demonstrate significant LBM accretion except where energy intake was excessive. He concluded that 'exercise and/or training has not been shown to markedly increase lean body mass'. His view was that only with the aid of steroids was appreciable gain achievable, reporting rates of gain of LBM in such body builders of up to 5·4 kg over 6 weeks, equivalent to about 0·3 g protein/kg per d. On the basis of our experience with 'natural' body builders, whilst muscle gain is difficult, nevertheless with appropriate

exercise regimens, including concentric contractions, significant muscle hypertrophy does occur but at a trivial rate as far as protein needs are concerned.

Physical activity can influence growth of LBM through stimulation of height growth in children (see Torun, 1993). The increase in height, over 6 weeks, of exercised children was 22 mm, but that of control children fed on the same diet was only 14 mm; the respective increases in urinary creatinine were 42 and 31 mg/d. Since the energy content of the weight gain by exercised children was calculated to be lower than that of controls (21 kJ/g v. 39 kJ/g) the authors concluded there was a 50% increase in LBM growth. For a 1-year-old child accreting N at 30 mg/kg per d (0·19 g protein), a 50% increase in growth amounts to an extra 0·095 g protein/d, which is still less than 10% of the DRV for a child of that age.

Thus, in the absence of steroid-induced growth, rates of protein accretion with exercise in adults are trivial accounting for up to 30 mg/kg per d, i.e. 3% of the mean adult protein intake in the UK (1·15 g/kg per d). Even for steroid abusers exhibiting maximal rates of growth of LBM, the accretion would only rise to 20% of these average intakes and of course would be a much lower proportion of the high-protein diet which such individuals generally take. In any case the increased energy expenditure that accompanies increased physical activity requires increased food intake and that would supply increased protein. In the UK at present, average energy intake by males is 1·39 times the resting metabolic rate (RMR) (Gregory *et al.* 1990). Our studies of body builders indicate an energy expenditure of 1·97 × RMR (Quevedo *et al.* 1991), requiring a 42% increase in energy intake for balance. The average protein intake by the adult male in the UK is 1·12 g/kg (Gregory *et al.* 1993), so that assuming that the protein–energy density in the increased food intake of the body builders is the same as that of the average UK diet (140 kJ/MJ total energy), they would have a protein intake of 1·58 g/kg, i.e. an extra 0·46 g/kg. Thus, the increased protein intake associated with satisfying the energy needs on a normal mixed diet will supply at least 50% more protein than the maximum rate of accretion recorded in the literature for steroid-induced weight gain. For normal non-drug-abusing athletes, the extra protein intake will be fifteen times the likely maximum rate of protein accretion.

MUSCLE DAMAGE AND REPAIR

Skeletal muscle most often generates force whilst shortening, i.e. lifting a weight, climbing stairs. However, muscle can also generate force during lengthening, i.e. lowering a weight. These two actions are called concentric and eccentric contraction respectively, and the distinction is important since eccentric exercise induces marked damage (Newham *et al.* 1983). During most physical activities it is likely that both concentric and eccentric exercise is performed; many will have experienced the aching leg muscles which follow a hill walk. This is almost certainly a consequence of eccentric contraction during the downhill phase (the performance of 'negative work'). After a marathon run there is evidence of extensive ultrastructural changes of the myofibrils, sarcotubular system and mitochondria which require major repair and regeneration over several months; this is achieved by satellite cells generating new myonuclei within the myofibres (Warhol *et al.* 1985). The consequences of this damage include several responses which may well influence protein metabolism and dietary needs (Table 1).

First, the damage, which includes a release of muscle enzymes such as creatine kinase

Table 1. *Influence of eccentric exercise on skeletal muscle*

1. Immediate and long-lasting ultrastructural changes
2. Release of muscle enzymes into plasma
3. Systemic inflammatory response
4. Accumulation of monocytes in muscle
5. Sustained increased protein turnover
6. Increased leucine oxidation and N excretion

(*EC* 2.7.3.2) into the plasma, provokes a systemic inflammatory response involving elevated plasma interleukin-1 (IL-1; Evans, 1986), increased neutrophils which migrate to the site of damage (Smith *et al.* 1989), and increased muscle IL-1b (Cannon *et al.* 1989), followed by accumulation of monocytes (Jones *et al.* 1986), and increased rates of muscle protein turnover which are a necessary component of the repair process. In animal models the increased protein turnover is intense and prolonged, and if the stimulus is sustained there is hypertrophy, as we showed many years ago in the weighted anterior *latissimus dorsus* muscle which supports the chicken wing (Laurent & Millward, 1980). More recent studies show increased expression and production of insulin-like growth factor-1 (IGF-1) within the muscle fibres (Yan *et al.* 1993), no doubt linked to satellite-cell activation which requires IGF-1 (Dodson *et al.* 1985). Athletes training for strength often deliberately include eccentric exercise in their programme in order to produce hypertrophy, although controlled studies of the relative influence of concentric, eccentric and isometric training on quadriceps hypertrophy show little difference over 12-week periods (Jones & Rutherford, 1987).

Some years ago we attempted to induce these changes in humans, measuring the incorporation of [1-^{13}C]leucine into human quadriceps muscle, but very few subjects were studied and the results were not consistent (Rennie *et al.* 1980). However, more recent studies in young men undertaking regular resistance exercise over a period of 12 weeks (Yarasheski *et al.* 1992) have shown a marked increase in quadriceps protein synthesis. This has also been observed in biceps muscles of subjects who had previously taken high-intensity resistance exercise (Chesley *et al.* 1992) with increases of [^{13}C]leucine incorporation of between 10 and 80% within 4 h which remained elevated for at least 24 h.

These changes may have widespread systemic influences on protein metabolism increasing demands for protein, although the net consequence of damage and subsequent repair has not been quantified (see Evans, 1993). In one study (Fielding *et al.* 1991), a single 45 min period of eccentric exercise at 80% maximum O_2 intake ($V_{O_2 max}$) resulted in increased leucine oxidation, together with increased whole-body protein degradation and urinary 3-methyl histidine (3-MeH) excretion which was still apparent 10 d after the exercise. Urinary N excretion on days 11–15 and leucine oxidation on day 10 were increased by 6 and 14% respectively.

MUSCLE CONTRACTION, AMINO ACID OXIDATION AND N LOSSES

Nature of any increased losses

From first principles there are several reasons why increased amino acid oxidation might be expected to accompany high levels of muscle contraction (Table 2). In fact there is

Table 2. *Influence of exercise on nitrogen and amino acid metabolism**

1. NH_3 production from amino acids via the purine nucleotide cycle
2. Increased use by muscle of amino acids (especially branched-chain) as fuels
3. Increased supply of amino acids with net muscle proteolysis
4. Increased amino acid oxidation and release from visceral tissues

* All effects vary with intensity, training, fuel availability, nutritional state and sex.

evidence that all these mechanisms may contribute, although much of the detail remains to be unravelled.

The purine nucleotide cycle and ATP homeostasis. The major metabolic imperative in muscle, driving most other metabolic changes during short-term exercise, is maintenance of the ATP:ADP ratio after ATP hydrolysis. Minimization of increases in AMP concentration during contraction is important to ensure maximal rates of conversion of ADP to ATP and AMP by the AMP-kinase (*EC* 2.7.4.3) reaction. AMP deaminase (*EC* 3.5.4.6) generates NH_3 and IMP from AMP, and the purine nucleotide cycle enables regeneration of AMP from IMP and aspartate (Lowenstein & Goodman, 1978). Recent work shows that AMP deaminase is activated during contraction by binding to myosin (Rundell *et al.* 1993). It is well known that muscle NH_3 production increases exponentially in relation to the exercise intensity but the source of the NH_3 is uncertain (see Rennie *et al.* 1994). It can arise simply as a result of adenosine deaminase (*EC* 3.5.4.4) activity at the expense of AMP, with stoichiometric production of IMP especially during high-intensity, short-term exercise. At lower work rates the complete operation of the purine nucleotide cycle results in the oxidative deamination of aspartate to resynthesize AMP, with aspartate replenished from amino acids oxidized in muscle. Clearly the capacity of any process to maintain myofibrillar ATP availability will influence the extent of AMP deamination, thus, NH_3 production will reflect availability of glycogen and other fuels for oxidative metabolism, especially free fatty acids (FFA) (Graham *et al.* 1991; Wagenmakers *et al.* 1991).

Amino acids as fuels within muscle. In addition to the involvement of aspartate in the purine nucleotide cycle, amino acid metabolism in muscle includes the transamination of alanine, aspartate, the branched-chain amino acids (BCAA) and glutamate, the oxidative deamination of glutamate, the oxidation of BCAA via the branched-chain α-keto-acid dehydrogenase (*EC* 1.2.4.4), and the synthesis of glutamine (see Rennie *et al.* 1994). Muscle's capacity for amino acid metabolism is demonstrated by the export of glutamine and alanine, in excess of their concentration in muscle protein, in amounts equivalent to the BCAA content of tissue protein (i.e. all the alanine-N and at least half the α-amino glutamine-N is derived from the BCAA). The high K_m of the BCAA aminotransferase (*EC* 2.6.1.42) reaction ensures that transamination is supply-driven and any BCAA from the blood will also contribute. Most importantly since human muscle has a high capacity for the complete oxidation of the BCAA it can use them as a fuel (Khatra *et al.* 1977; Elia & Livesey, 1981). Furthermore, in human muscle the transamination and oxidation of BCAA is increased in exercise, no doubt as a result of the progressive activation of the branched-chain α-keto-acid dehydrogenase as the muscle glycogen concentration falls (Wagenmakers *et al.* 1989, 1991). FFA will also influence BCAA oxidation since increasing the availability of FFA during exercise at
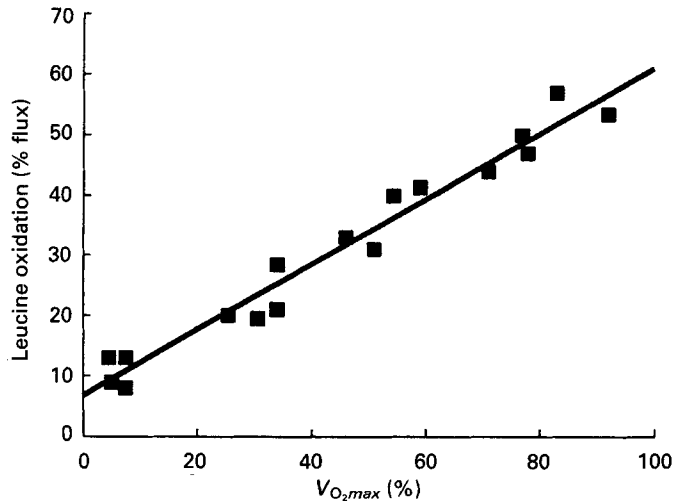
Fig. 3. Influence of increasing levels of physical activity on a bicycle ergometer on [^{13}C]leucine oxidation (M. J. Rennie, D. Halliday and D. J. Millward, unpublished results). V_{O_2max}, maximum O_2 intake.

80% of V_{O_2max} inhibits muscle ammonia, glutamine and alanine release (Graham *et al.* 1991), possibly through inhibition of leucine transamination and oxidation by long-chain FFA (Frick & Goodman, 1980).

In humans exercising at 40% V_{O_2max} there is a sustained increased muscle uptake of glutamate and the BCAA and production of alanine and glutamine. After 4 h the net flow of amino acids from muscle to liver was shown to be contributing about 18% of hepatic gluconeogenesis (Felig & Wahren, 1971). Thus, increased leucine oxidation during exercise might be expected, and this has indeed been observed. We and others have shown with [^{13}C]leucine that whole-body leucine oxidation is increased in exercise in proportion to the relative intensity (Lemon *et al.* 1982; Millward *et al.* 1982) (see Fig. 3), and studies using the human forearm demonstrate conclusively that the increase does represent events in muscle (see Rennie *et al.* 1994). Indeed, the circulating keto acid of leucine (α-ketoisocaproate) is actually taken up during forearm exercise and this too is oxidized.

With high-intensity exercise this increasing BCAA oxidation in muscle is associated with a marked increase in alanine output (e.g. 300% increase at 55% V_{O_2max} and 900% at 80% V_{O_2max}, Eriksson *et al.* 1985). The increase in glutamine release is less and in any case is partly at the expense of intramuscular glutamate (Eriksson *et al.* 1985; Katz *et al.* 1986; Henriksson, 1991). Trained athletes tend to oxidize more fat and less carbohydrate than the general population; therefore, because amino acid oxidation roughly matches glycogen utilization, alanine production (an indicator of amino acid oxidation) by trained athletes is less (see Rennie *et al.* 1994). However, it is likely that overall N flow out of muscle during exercise exceeds amino acid oxidation since it includes a depletion of the intramuscular glutamine pool which is large (>20 mM in human muscle). In our own studies the fall in intramuscular glutamine concentration during a 3·5 h treadmill exercise

(Rennie *et al.* 1981*a*) accounted for a large component of the overall N loss induced by the exercise (see Millward *et al.* 1982).

Increased net muscle protein degradation during contraction. High-intensity exercise which depletes muscle ATP and induces anoxia can induce negative protein balance with increased concentrations of BCAA through a marked inhibition of protein synthesis and lesser inhibition of proteolysis. This is observed in the perfused rat hindlimb (Bylund-Fellenius *et al.* 1984) and in human muscle in hypoxia (Rennie *et al.* 1983). We reported a decreased concentration of 3-MeH indicative of decreased proteolysis in muscle biopsied immediately on termination of a treadmill exercise (Rennie *et al.* 1981*a*), and more recent studies with [^{13}C]leucine have confirmed the reduced protein synthesis and degradation in human forearm (Rennie *et al.* 1994). In contrast, Henriksson (1991), studying bicycle exercise at a similar rate, found a small increase (25%) in muscle 3-MeH. Those studies reporting increased urinary excretion of 3-MeH are difficult to interpret in terms of the tissue sources of the 3-MeH especially given the evidence of increased net protein breakdown in gut during exercise in the dog (see below). However, our own studies in rats which were not exhausted on a treadmill failed to indicate any inhibition of protein synthesis (Bates *et al.* 1981) so that any net protein catabolism during the exercise may only occur during exhaustion.

Increased net protein degradation in visceral tissues during exercise. One of the main metabolic consequences of physical activity is the change in blood flow from the splanchnic bed to the periphery. Reduced perfusion of the liver together with the increased glucagon and fall in insulin are likely to result in an increase in autophagy, an overall protein catabolic state certainly well documented in the rat (Dohm *et al.* 1987). In exercising dogs (Wasserman *et al.* 1991) exercise causes the gut to become a net exporter of amino acids as a result of increased net protein degradation. Thus, substantial amounts of the substrates for hepatic gluconeogenesis (e.g. alanine) and oxidation by muscle (e.g. BCAA) may be originally derived from gut protein.

Extent of any increased losses

Nitrogen balance studies. On the basis of the previous discussion it is clear that the potential exists for increased amino acid oxidation in exercise through several mechanisms. However, it is also the case that the extent of any increased oxidation depends on several variables, namely: (1) exercise intensity (Fig. 3); (2) the acute availability of energy supply in terms of either glucose (Davies *et al.* 1982) or FFA (Graham *et al.* 1991); (3) previous nutrition, as indicated by the larger stimulation of leucine oxidation by exercise in the fasted compared with the postabsorptive state (Knapik *et al.* 1991); (4) training; athletes produce less alanine across muscle (see Rennie *et al.* 1994); (5) sex; it appears that, in comparison with men, women oxidize a higher proportion of lipids, sustain a smaller fall in muscle glycogen and exhibit a lower N excretion during exercise (Tarnopolsky *et al.* 1990). This all points to the unlikelihood of identifying any fixed stoichiometry between muscle activity and amino acid balance on the basis of current understanding.

Whilst all these reports of increased leucine oxidation and efflux of muscle N might be taken as firm evidence of an effect on N balance, Wolfe and co-workers (Wolfe *et al.* 1984; Wolfe, 1987) argue against such a conclusion. They observed increased leucine oxidation in response to exercise at 40% V_{O_2max} but no change in urea production or
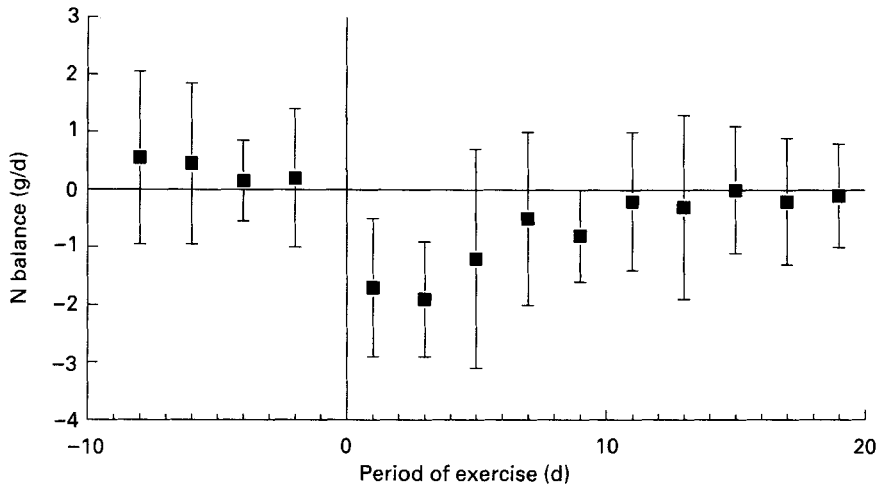
Fig. 4. Adaptation of nitrogen balance to physical activity (from Gontzea *et al.* 1975). Values are means and standard deviations represented by vertical bars.

excretion. They argue for selective BCAA oxidation with retention of the remaining amino acids in hepatic proteins which are low in BCAA. In support of this argument they report increased synthesis rates of fibronectin and fibrinogen during exercise, suggesting that about one-sixth of the amino acids leaving muscle could be taken up by fibrinogen alone (see Wolfe, 1993). Whilst this is an interesting suggestion which could occur at the somewhat low levels of activity (40% V_{O_2max}) in their studies, it seems unlikely at higher levels when both liver and gut are in a net catabolic state, as discussed previously.

In any case there is ample evidence in the literature of increased N excretion in response to exercise. In our own studies of the acute impact of exercise on whole-body protein and amino acid metabolism (Rennie *et al.* 1980, 1981*a*) we showed a substantial exercise-induced increase in blood urea concentration during the exercise and an increased urea excretion after exercise when renal filtration, which falls during exercise, is restored to normal. Demonstration of the prompt increase in leucine oxidation in this and subsequent studies (Millward *et al.* 1981; Rennie *et al.* 1981*b*, 1994) confirmed the exercise period as the source of the increased urea production.

The studies conducted in Romania some years ago remain amongst the most convincing N balance studies (Gontzea *et al.* 1975; Fig. 4). These were careful studies of untrained individuals maintained on energy intakes 10% in excess of their measured expenditure throughout the studies which involved six daily 20 min bicycle exercises. Attention was paid to collecting all sources of N excretion including sweat which otherwise can lead to serious underestimation of losses (Lemon & Nagle, 1981). On an intake of 1 g protein/kg per d, which maintained zero balance in the rested state, the exercise induced an immediate negative N balance. However, there was a 90% reduction in the excess N excretion over the subsequent period until eventually zero balance was restored. The adaptation of N balance was accompanied by an improved energetic efficiency, with lowered pulse and respiratory rates. The authors concluded from their
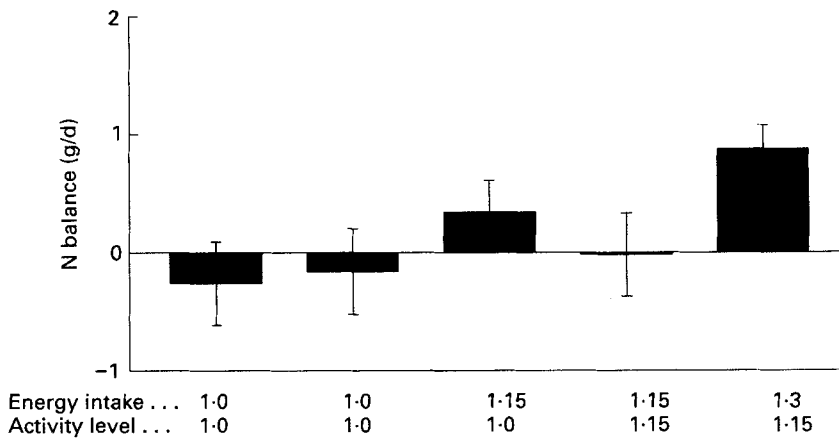
Fig. 5. Nitrogen balance with increasing energy intake and physical activity (determined as that which allowed energy balance (=1)) (from Butterfield & Calloway, 1984). Values are means and standard deviations represented by vertical bars.

studies that habitual physical activity would not increase requirements for protein in fully trained individuals when energy needs were met.

The studies of Butterfield & Calloway (1984) demonstrate an important influence of exercise on N balance in subjects on lower than average protein intakes. These studies (Fig. 5) involved careful regulation of energy intakes and expenditure during successive 16 d balance periods and compared the influence of an exercise programme on N balance at zero energy balance and at a fixed level of energy supplementation. N balance was improved by the exercise in each case and the changes in LBM were by and large consistent with the N balance data. Whilst it is well known that inactivity associated with bed rest results in loss of LBM, these studies are unique in showing the importance of physical activity for optimization of dietary protein utilization in normal adults.

In contrast to these studies two other groups have reported short-term N balances in athletes undertaking physical activity which imply an increased protein requirement. Meredith et al. (1989) reported N balances in young and middle-aged runners randomly given three levels of dietary protein. Both groups achieved balance at 0·94 g/kg per d, i.e. about 17% above the current US recommended dietary allowance (Food and Agriculture Organization/World Health Organization/United Nations University, 1985). However, it would have been more informative if sedentary controls with the same dietary background had also been studied. Given the wide range of values for protein requirement determined in short-term balances (Food and Agriculture Organization/World Health Organization/United Nations University, 1985) it is not certain whether the protein intake for zero balance in the active groups studied was in fact elevated compared with the sedentary members of the population studied.

Tarnopolsky et al. (1988) reported N balances in sedentary controls, body builders and runners, measured at their habitual intake and one other intake (higher for runners, lower for body builders and controls). Regression to zero balance indicated protein requirements of 0·73, 0·82 or 1·37 g/kg per d for controls, body builders and runners respectively. The concern with these, as with most balance studies with high protein
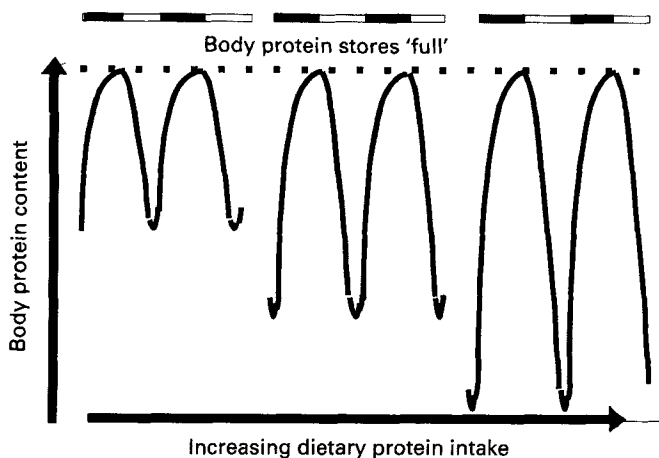
Fig. 6. Schematic representation of the diurnal changes in body protein with increasing protein intakes.
(■), Feed; (▭), fast.

intakes, is with the very large apparent positive balances observed, i.e. up to 12 g N/d on the highest protein intakes. This is equivalent to approximately 500 g lean tissue/d, an impossibly large gain. Also, the direction of diet change seems to be associated with the outcome; this raises concern that the lack of adaptation to the new intake may have influenced the zero balance intercept. In fact, excessive losses when the change is to the lower intake, and excessive gain when it is to the higher intake, would increase the slope of the regression and increase the zero balance intercept in each case.

Clearly these various studies do not completely resolve the issue of the extent of any obligatory increased protein needs associated with exercise but they do suggest that with sufficient energy and with training, physical activity has no effect on N balance other than improving it compared with complete inactivity.

PHYSICAL ACTIVITY, HIGH-PROTEIN DIETS AND PROTEIN REQUIREMENTS

As indicated at the start of the present review an essential feature of N homeostasis is the regulatory response of amino acid oxidation and N excretion to external factors of which, in addition to physical activity, the extent and daily pattern of dietary protein intake is the most important influence. In our studies we have explored the relationship between protein intake and N homeostasis within the context of a diurnal cycle of protein balance, assuming that body protein varies with intake according to the scheme in Fig. 6. The first component of this scheme is that regulation of body protein occurs to maintain a 'body-protein-full' state which is a function of height and which cannot be exceeded by ordinary dietary means. This assumption is based on the growth pattern in normal children and the phenomenon of catch-up growth (Ashworth & Millward, 1986), and on the relative stability of the LBM in adults (Forbes, 1985). One mechanism, briefly described elsewhere (Millward, 1989), which could limit protein deposition in skeletal muscle is based on the idea that the volume of myofibres is limited by the inelastic and relatively fixed collagen connective tissue endomysial 'bag' surrounding each fibre. Physicochemical factors would limit protein deposition in this fixed volume and 'bag'
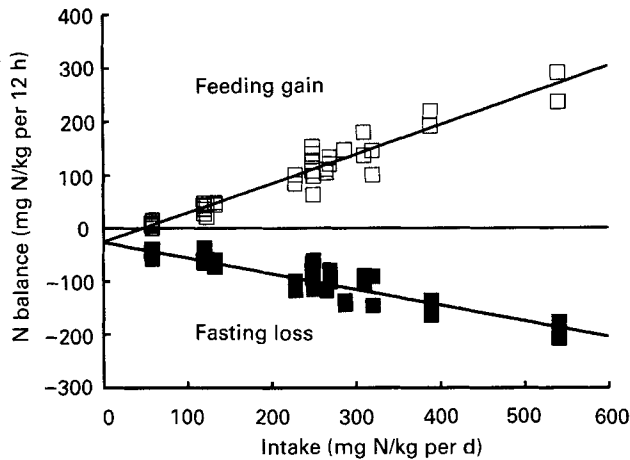
Fig. 7. Diurnal cycling of body protein with increasing protein intakes (from Price *et al.* 1994).

enlargement would rate-limit muscle growth. Clearly in the context of either body building or obesity this control mechanism can be overridden in some circumstances (bag rupture) so that the upper limit can be exceeded.

The second component of the scheme in Fig. 6 is that the amplitude of the diurnal cycling of body N, i.e. fasting losses and matching postprandial gains, varies with the protein intake, as shown over a very wide range of protein intakes by both N balances (Fig. 7) and [^{13}C]leucine oxidation studies (Price *et al.* 1994). On this basis the maintenance protein requirement can be defined as a dietary intake which allows sufficient fed-state gain to replace night-time losses. Adaptation to a new intake requires adjustment of both postabsorptive losses and of the efficiency of postprandial gain. In fact, individuals accustomed to very-high-protein diets who change to a lower intake are unable to achieve sufficient postprandial gain to replace postabsorptive losses, and maintain a daily negative N balance for more than 1 week (Fig. 8; Quevedo *et al.* 1994). This slow adjustment to a new level means that any acute reduction in protein intake is associated with considerable losses. In other words individuals with a goal of expanding their LBM and consuming high-protein diets cannot afford to relax this aspect of their dietary regimen without losing body protein and possibly reversing any gain achieved.

Fig. 9 shows another potentially adverse consequence of high-protein diets during training. In subjects given two levels of protein for a 2-week period before a treadmill exercise at 50% V_{O_2max} for 2 h with or without glucose, leucine oxidation in the first hour was higher in the subjects given the high-protein diet (J. L. Bowtell and M. J. Rennie, unpublished results). Thus, in the context of the metabolic scheme in Fig. 1 regulatory oxidative losses associated with physical activity are not only adaptive with training but are also variable with diet, increasing with protein intake.

CONCLUSIONS

The nutritionally relevant question is whether physical activity increases the need for dietary protein. Clearly growth of the LBM can be ignored. It does appear that, in the
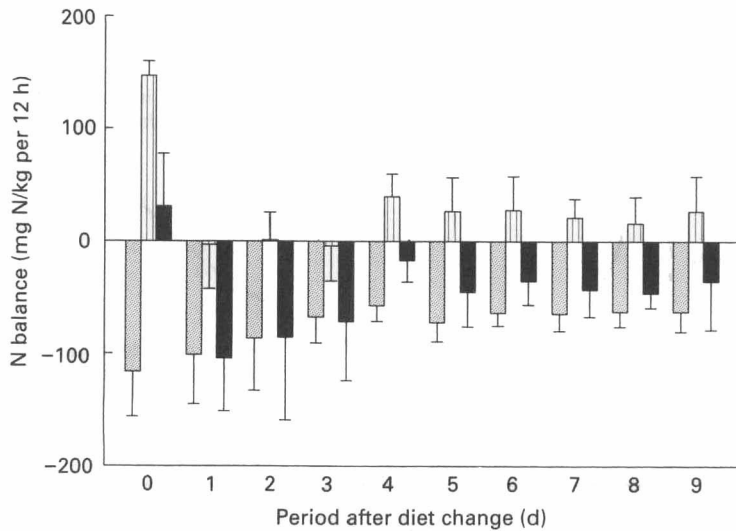
Fig. 8. Adaptation of the diurnal changes in N balance to a reduction in protein intake from 2 to 0·75 g/kg per d. The changes in body N are shown as measured during successive 12 h periods of fasting (□) and feeding (□) and as daily overall balances (■) (from Quevedo *et al.* 1994). Values are means and standard deviations represented by vertical bars.
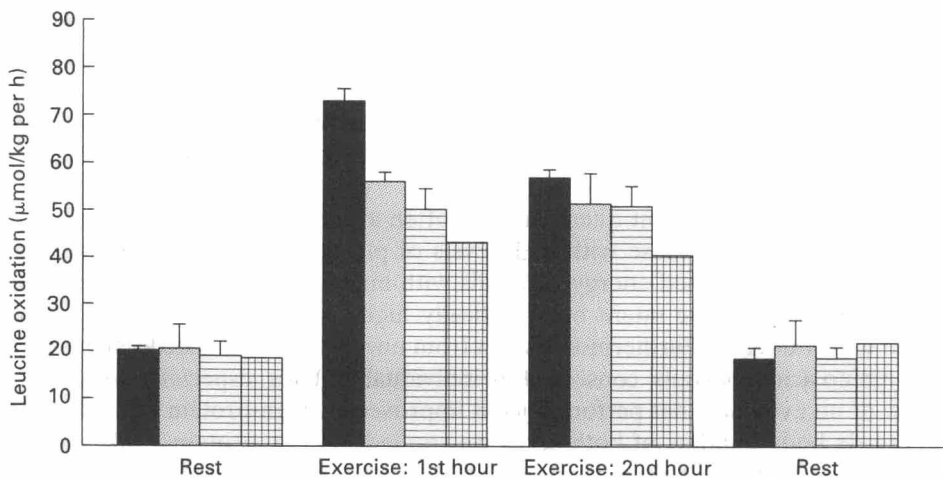

Fig. 9. Leucine oxidation during treadmill exercise in subjects given one of four diets (high-protein, ■; high-protein plus glucose, □; low-protein, □ or low-protein plus glucose, □) for 2 weeks before the study (J. L. Bowtell and M. J. Rennie, unpublished results). Values are means and standard deviations represented by vertical bars.

untrained, bouts of unaccustomed exercise do provoke N losses in variable amounts according to their type, intensity, and immediately previous nutritional state. The influence of chronic malnutrition on exercise-induced amino acid oxidation and N balance has not to our knowledge been extensively explored but is clearly a most
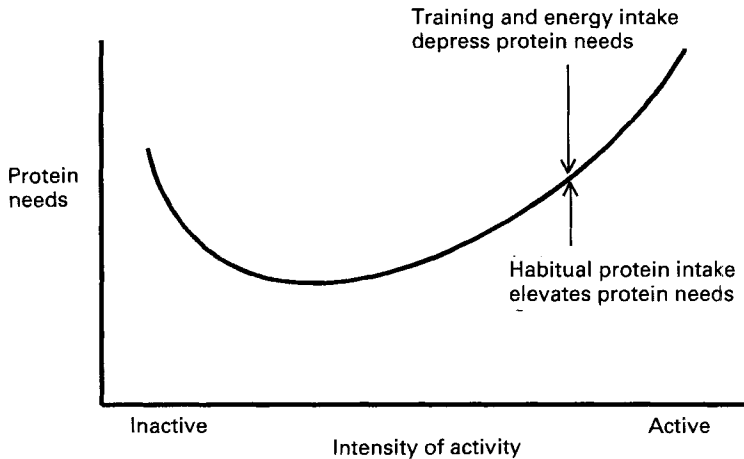
Fig. 10. Relationship between physical activity and protein needs. Protein needs are those allowing N balance. The extent of any increase in protein needs with increasing physical activity appear to be variable, any increase being minimized by training and adequate energy supply but increasing with the habitual protein intake.

important question. However, in the appropriately trained individual with adequate energy supply, N losses associated with physical activity may be minimal or even less than in sedentary individuals (Fig. 5). From the evidence available, the relationship between protein needs for overall N balance and physical activity would appear to be represented by a U-shaped curve, with needs increasing in both the inactive individual and with intense activity, although the extent of any increase with activity is reduced by both training and energy provision but may be increased by increasing dietary protein (Fig. 10).

The physiologically relevant question most often asked is whether increasing protein intakes are of benefit to those with high levels of physical activity. As already stated, satisfaction of the increased energy demands with mixed food will in any case increase protein intakes. For those intent on specifically high-protein diets, we have identified potential disadvantages. Furthermore, apart from unsubstantiated anecdotal evidence of various amino acid 'cocktails' consumed by individual athletes, especially body builders, we know of no evidence that performance is improved by high-protein diets. In a group of twenty-six body builders of both sexes we studied, dietary protein intakes averaged 1·93 (SD 0·46) g/kg per d, values ranging from 0·63 (a lacto–ovo vegetarian) to 3·05 g/kg with diets of protein–energy densities ranging from 82 to 280 kJ/MJ total energy, with no discernible impact. However, as is often the case with the nutrition literature the key experiments are to be found described in the classic texts written decades ago. Chittenden (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/kg per d. Extensive measures of strength were made. Over the 5 months their strength increased on average by 35% (see Table 3), coupled with a fall in perceived fatigue. Similar studies with soldiers over 6 months resulted in an 85% average increase, although these were untrained at the outset so that their final strength

Table 3. *Strength of male athletes before and after a 5 month period of a reduced-protein diet**

Athlete no.	Total strength†		
	Initial	Final	Increase %
1	4913	5722	16
2	6016	9472	57
3	5993	8165	36
4	2154	3983	85
5	4584	5917	29
6	4548	5917	30
7	5728	7135	23
8	5351	6833	28
Mean			38

* Exclusion of most dietary meat and reduction of protein intake by 'more than 50%' to about 55 g protein/d over 5 months.

† Sum of fifteen distinct tests of measured strength and work performed in various exercises (see Chittenden, 1907).

was similar to the athletes at the start of their experiment. Chittenden (1907) concluded that his experiments 'afford reasonable proof of the beneficial effects of a lowered protein intake upon the muscular strength of man,' and that 'man can profitably maintain nitrogen equilibrium and body weight upon a much smaller amount of proteid food than he is accustomed to consume.' In the context of minimal and optimal protein requirements, Chittenden (1907) defined an optimal daily requirement of about 0·75 g/kg, equivalent to the current UK DRV (Department of Health, 1991) and significantly less than the average UK intake. Chittendon's (1907) views were controversial at the time and have been given less prominence than they deserve in the intervening years of continued controversy. However, the findings speak for themselves.

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