A critical review of recommendations to increase dietary protein requirements in the habitually active

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Abstract

Some scientists and professional organisations have called for an increase in dietary protein for those who reach a threshold level of exercise, i.e. endurance athletes. But there are individual scientists who question this recommendation. Limitations in the procedures used to justify changing the recommended daily allowance (RDA) are at issue. N balance has been used to justify this increase; but it is limiting even when measured in a well-controlled clinical research centre. Experimental shortcomings are only exacerbated when performed in a sports or exercise field setting. Another laboratory method used to justify this increase, the isotope infusion procedure, has methodological problems as well. Stable isotope infusion data collected during and after exercise cannot account for fed-state gains that counterbalance those exercise losses over a 24 h dietary period. The present review concludes that an adaptive metabolic demand model may be needed to accurately study the protein health of the active individual.

Key words: Nitrogen balance: Stable isotope tracer infusion: Protein recommended daily allowance for athletes: Dietary protein needs of athletes: Endurance athletes

Introduction to the scientific dialogue

Protein and amino acids only contribute 2–3 % of the total energy needs during endurance exercise and it is unclear whether this leads to an increased demand for these nutrients[1–3]. The debate as to whether dietary protein should be increased in those who habitually train has been ongoing. One position paper published in the year 2000 recommended an increase in dietary protein for the endurance athlete; however, this statement was revised in 2009 to indicate that no increase was necessary[4,5]. This issue has transcended the point where it is an academic discussion because it has affected a misunderstanding in the lay public about dietary protein health[2]. Nutrition supplement stores and on-line web sites consistently rank protein pills and powders among the top dietary aids sold[6]. These supplements are widely used but there appears to be a general misunderstanding about them. Young individuals are one age group that consumes and misuses supplemental amino acids and protein as nutritional aids[7–9]. A meta-analysis pooled fifty-one studies of 10 000 high-school athletes and reported that nearly half the sample consumed supplements (46 %)[7,8]. But only ten out of 328 college athletes (3 %) were correctly able to identify the recommended daily protein energy needed for health[9]. About half the sample believed protein was the main energy source for muscle and one-third thought that protein supplementation was necessary to improve athletic performance[9]. These findings attest to a misunderstanding of exercise and protein health among those who exercise.

One serious problem with recommending an increase in dietary protein is that there are training-induced adaptations in amino acid economy that have not been accounted for in the laboratory methodologies used. In addition, modification of the N balance procedures to a sports field setting may be convenient[10–14] but it presents numerous methodological shortcomings. Limitations of N balance, even when performed in a well-controlled clinical research setting, are known, and 25 years ago the nutritional scientist Dr Vernon Young[15] cautioned that: '... nutritional balance studies have provided useful and important data about human protein and amino acid requirements, as well as about changes in protein and amino acid metabolism under various pathophysiological states. The technique is likely to continue to serve as a tool in nutritional studies, but its major limitations should be better appreciated. Reliance on this classical method as a principal procedure in studies of human nutrient requirements cannot be justified and, indeed, could be misleading.'

Although this quote was not directed to the sports nutrition community, the chance for making misleading recommendations using N balance data are just as relevant. Misunderstandings in the general public about protein nutrition attest to the need for a cautionary tone when giving dietary advice. The present review outlines this controversy and focuses on the experimental limitations in studying protein needs of the endurance athlete. It concludes that the recommendations to raise the protein recommended daily allowance (RDA) above that recommended for the healthy, sedentary adult have not been justified.

Exercise studies using field-based metabolic balance

Our ability to study whole-body protein metabolism is limited to a few procedures; one of the oldest is the N balance technique. N balance measurements require the collection of all N leaving and entering the subject in order to assess N equilibrium[16]. N output (N leaving the body) is determined from the N in sweat, urine, faeces and other miscellaneous routes of elimination. N intake (N entering the body) is determined by dietary analysis. Equilibrium (positive or negative N balance) is calculated from these N intake and output data. Professor John Waterlow[16] published an extensive review of N balance in *Nutrition Research Reviews* many years ago. His review makes clear that N balance is a complex metabolic mystery that we do not fully understand. How changes in dietary protein translate into alterations in urea cycle activity still perplexes nutritional scientists. Recent research indicates that the urea cycle, comprised of five enzymes, requires additional enzymes and mitochondrial amino acid transporters to function and that major regulators of the liver's urea cycle enzyme expression include glucagon, insulin and glucocorticoids[17]. Urea N production can exceed N intake – and only through a transfer of the urea N produced to the colon, where it is hydrolysed into NH_3, can N balance be attained. Urea kinetics measured during exercise demonstrated that there are increases in branched-chain amino acid oxidation without a simultaneous increase in the rate of urea production[18].

Current dietary recommendations are that dietary protein should equal the intake necessary to maintain N equilibrium as measured with multi-level N balance studies. Although this procedure allows for the determination of positive or negative N balance, it does not reveal the specific ways in which a new state of balance has been achieved. The equilibrium measure known as the 'estimated average requirement' can be expressed as a reference value (mean + 2 standard deviations). This reference or RDA can be used prescriptively to ensure that there will be a low risk of consuming less protein needed for optimal health[19,20]. The protein RDA for endurance athletes was amended in response to N balance analyses some years ago[4,21–25]. It was increased from 0·8 g/kg per d as recommended for the healthy adult to 1·2–1·4 g/kg per d for the endurance athlete. Adoption of this nutritional change is now commonplace in sports nutrition textbooks[26–30] but it has fallen short of scientific standards needed for its defence[1–3].

N balance is far from an ideal laboratory procedure. When applied in a field setting the experimental limitations are accentuated and the data accuracy eroded because of a lack of control over the research environment. Fig. 1 outlines the measurements used in these exercise studies: sweat and/or urinary urea N (accounting for about 90 % of N excretion). But other routes of N output such as faecal, dermal or miscellaneous losses were not measured or were indirectly estimated[10–14]. Faecal N could have been detectable because not all protein is completely absorbed and N not reabsorbed is secreted into the gastrointestinal tract. Also there was a lack of measurement of urinary ammonia, uric acid and creatine which are substantial contributors to N output[19,31]. Renal blood flow and glomerular filtration rate decrease during prolonged exercise which further compounds the incomplete measure of N output in these balance studies[32]. Prolonged exercise can enhance urea reabsorption but it is unknown how these changes in kidney function influence urinary markers of whole-body protein catabolism[33]. Lastly, the slow turnover rate of the urea pool makes it difficult to determine the length of the collection period needed to ensure measurement accuracy[18].

N balance studies of endurance athletes have been used to justify an increase in dietary protein but they have had methodological issues on the N input side of the balance equation as well. Alterations in protein intake can take weeks, if not months, to attain a new steady state in N metabolism[34]. When N intake is above minimum requirements (as is likely in many athletes), N balance remains unrealistically positive for a long time[35]. This is a methodological issue that has not been adequately addressed in sports nutrition experiments. Studies indicate that an adequate dietary control would require at least
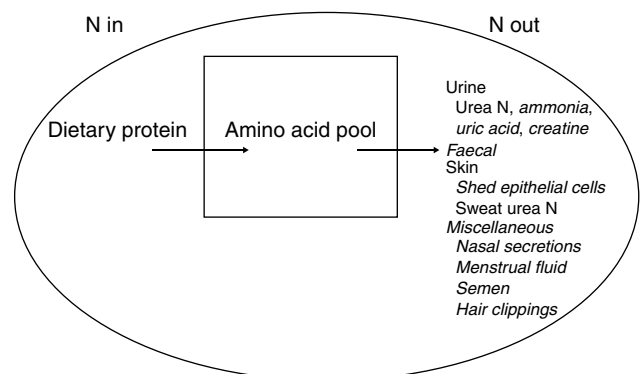

Fig. 1. The nitrogen balance model for determining protein requirements as adapted from Fukagawa & Fisher[19]. Note those variables that are italicised have not been measured with the field-based nitrogen balance (some studies have estimated these).

3–4 weeks and most have used an abbreviated dietary control period of 3–12 d. This dietary approach is still being employed in N balance experiments[36]. Also field-based metabolic balance must by its very nature employ food diaries or dietary recall to estimate nutrient intake, a procedure known to produce issues with memory gaps and inconsistencies in judging portion size[37,38]. A few studies have used prepackaged meals as a way to control for protein intake; but subject compliance is difficult to ensure. The accuracy of measuring N input during the balance measurement can be highly dependent on the athlete's compliance. Lastly, because protein intake is provided and monitored in the field as opposed to a clinical research centre, software analysis of nutrient content provides an additional step for computational error.

Peripheral issues make an impact on the accuracy of field-based N balance measures as well. Criticisms of adapting N balance to the field setting include an inability to control for: state of aerobic training, steady-state energy balance, and habitual training intensity[39]. Previous research indicates that dietary protein efficiency adapts and N retention improves with aerobic training[40]. This previous experiment reported that protein requirements were reduced in the endurance athlete[40] because of these training-induced adaptations in whole-body protein metabolism. This study underscores that experimental accuracy of field-based N balance measures is difficult to ensure. In addition, it highlights the impact of energy intakes on N balance and underscores that increases in energy intake in and of itself can improve both N retention and dietary protein efficiency[39,40]. Munroe highlighted some 30 years ago that N balance is extremely sensitive to energy intakes[41] and there are reports of fasting subjects reducing N loss with just small amounts of non-protein energy[42]. Both dietary carbohydrate and fat reduce net whole-body protein use. Increases in daily energy intakes will reduce the need for protein to maintain N balance. In fact, 30 % of the variance in N balance can be accounted for by energy intakes[43]. Cross-sectional comparisons between sedentary and well-trained subjects have not (and probably cannot) match for energy consumption. Because athletes require greater energy than their sedentary counterparts, field-based N balance studies will always be problematic. Another peripheral issue to the accuracy of N balance applied to the sports setting is that hydration influences urinary urea concentration in a manner independent of whole-body tissue protein breakdown[44]. High dietary protein intakes increase urea excretion (renal solute) that in turn heightens the need for water[44]. Finally, there are sex differences in the contribution of protein and amino acids to whole-body energy needs[45–48] and these have not been included in the protein RDA recommendations for the habitually active.

One nutritional scientist cautions that making generalisations about protein health from N balance oversimplifies a very complex biochemical process[20] and the reproducibility of the N balance method itself is poor[15]. These limitations exist even when performed in a well-controlled clinical research centre where test kitchens can strictly control for dietary intakes, physiological samples can be collected in total, and the activity level and energy balance of the subject can be strictly monitored and assured.

To summarise, the recommendation to increase protein intake, as determined from N balance, has had serious methodological problems on both the input and output sides of the equation. The procedure is a 'black-box' technique that does not allow for an assessment of the metabolic pathways involved in whole-body amino acid and protein metabolism. The estimates that this procedure has provided have often been unrealistic[2,44]. For instance, one sport scientist calculated that athletes placed on 2·5 g protein/kg body weight per d had a positive N balance of 17 g each day. But this would be an impossible lean tissue gain of 110 kg/year[2,13,44]. Some argue that errors such as these are due to an exercise-induced expansion of the circulating urea pool as well as a physiological limit to the rate of urea excretion[2].

Exercise tracer studies

The accuracy of N balance can also be questioned because other biochemical procedures have provided contradictory experimental results. Contemporary studies of stable isotope tracers such as glycine and leucine ([^{15}N]glycine and [^{13}C]leucine) have been used to study whole-body protein metabolism in the endurance athlete. Glycine tracer methodology can be useful because a shorter experimental time will be necessary as NH_3 is the endproduct sampled. This procedure has been standardised and validated[49] and collection periods of 12 h have been found to be suitable to study whole-body protein metabolism[50,51]. A urea tracer, [^{15}N]urea, has also been studied but it is difficult to determine a suitable collection period because of the slow turnover rate of the urea pool. Whole-body protein turnover measured with the glycine isotope showed no difference between young and middle-aged endurance trained men or between sedentary men (although differences in N balance were found)[11]. No training effect was reported for protein turnover when glycine tracer experiments were conducted in young female gymnasts engaged in intensive training[52]. This tracer methodology was also used to study an unassisted Antarctic crossing that required extreme dietary energy intakes. These subjects maintained protein synthesis throughout this ultra-endurance event[53].

A label of the essential amino acid leucine has also been used to study whole-body protein metabolism during endurance exercise and a comparison between the glycine and leucine tracer techniques has been performed[54]. A stable isotope infusion of the branched-chain amino acid [1-^{13}C]leucine indicated that there was no difference in amino acid oxidation between rested, fasting endurance

trained athletes when compared with age-, sex- and body weight-matched sedentary controls[55,56]. A 6 h study of leucine oxidation during resting conditions found that the athletes and sedentary controls had similar oxidation rates when expressed per unit of whole-body muscle mass[55]. This cross-sectional comparison suggests that habitual exercise does not alter resting protein needs. But when protein requirements are determined it will also be important to determine if there are physiological differences during exercise training.

Most athletes train a few hours each day and their need for protein will be altered if there is a prolonged increase in the use of this macronutrient for energy requirements. Branched-chain amino acid oxidation during aerobic exercise has been studied in numerous experiments[18,56–62] and a graph of these can be found in Fig. 2. During prolonged exercise, leucine oxidation increases approximately two-fold over resting needs and this has been modestly correlated with oxygen consumption (exercise V_{O2}; R 0·69). This statistical relationship suggests that amino acid use during exercise can be regulated by many factors other than training intensity[63] including: carbohydrate availability[64,65], NADH:NAD$^+$ ratio, acyl-CoA:CoA ratio, ketoacid availability[66] and β-adrenergic receptor stimulation[58]. Also important to the athlete's requirement for dietary protein is the response during exercise recovery in which there are reports of a drop in leucine oxidation below resting values[57,67].

Another important stable isotope tracer study[68] highlighted that moderate exercise at energy balance does not affect leucine equilibrium or N retention over the subsequent 24 h period. The subjects were able to stay in leucine balance and had no additional protein need after undergoing two, 90 min exercises during both a fed and a fasted state. This underscores that acute increases in amino acid and protein use during exercise cannot be extrapolated to indicate that there will be an increased dietary requirement[68]. It also indicates that the protein requirement (protein RDA) can only be determined with an appropriate research design. The research design must account for both protein losses and gains throughout the diurnal cycle. Although this study reported that amino acid oxidation increased during exercise, this increase was offset throughout the daily feeding cycle.

There are other experiments that have measured whole-body amino acid and protein metabolism using research designs that allow for an assessment of training adaptations. These designs include: a cross-sectional comparison of trained and sedentary individuals as well as a brief exercise intervention. The cross-sectional comparison showed leucine oxidation to be similar in the well-trained athlete when compared with the untrained, sedentary control exercising at similar moderate intensities (half of maximal capacity). Amino acid oxidation expressed in relative terms, i.e. per unit of oxygen consumed (or fat-free mass) did not differ between endurance-trained (28 μmol/kg body weight per litre oxygen) and sedentary control subjects (34 μmol/kg body weight per litre oxygen)[55]. This study does indicate that there are training-induced adaptations that lower the need for branched-chain amino acids, an observation consistent with the study by Butterfield & Calloway that physical activity enhances protein efficiency[40]. An exercise intervention is another research design that can be used to study protein requirements for the habitually active[56]. Subjects engaged in a 38 d training programme had a reduction in the oxidation of branched-chain amino acids and, importantly, a reduction in the regulatory enzyme in this oxidative pathway. Again, these data indicate that training-induced metabolic adaptations will decrease the need for whole-body amino acids and protein[56]. Both of these experiments contradict the recommendation to increase dietary protein in the habitually active individual. Instead, they indicate that small amounts of protein are used during exercise and that with training the need for this nutrient is down-regulated. Aerobic training causes an enhanced metabolic efficiency of protein used at the whole-body level. Table 1 [18,55,69,70] outlines that the absolute amount of whole-body protein oxidised during a 1 h
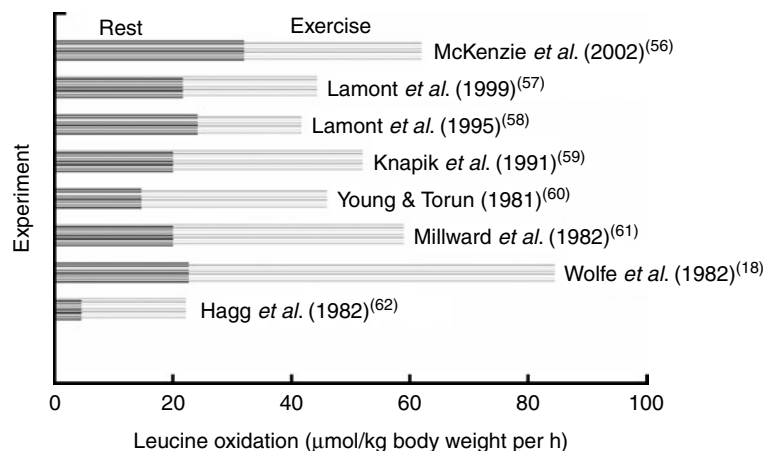

Fig. 2. Rate of leucine oxidation during acute endurance exercise. Redrawn using data from several sources[18,56–62].

Table 1. Whole-body protein oxidised during 1 h of moderate endurance exercise[70]*

Leucine oxidation of well-trained endurance athlete who is 77 kg body weight

52 μmol leucine oxidised/kg per h of exercise[55]

Assuming 590·0 μmol leucine in each g of whole-body protein[18]

This cyclist would oxidise 6·77 g of whole-body protein during a 1 h workout

(52 μmol × 77 kg × 1 h = 6·77 g)†

By contrast, the daily need (using recommended changes in protein RDA from 0·8 to 1·4 g/kg) would require this same athlete to consume an additional 46·2 g protein per d

(77 kg × 0·8 g/kg = 62 g/kg → 77 kg × 1·4 g/kg = 108 g/kg)

RDA, recommended daily allowance.

* The limitations of using leucine as a representative tracer of whole-body protein are acknowledged and discussed in depth elsewhere[70].

† This equals the amount of protein in one hard-boiled egg or one-third of a cup of cashew nuts[69].

endurance exercise at half of maximal capacity is quite small and amounts to the protein in one egg or a small serving of cashew nuts. Some have argued that low to moderate exercise does not negatively make an impact on protein balance but the elite athlete engages in intense training and therefore will be at risk[31]. The subjects used for this computation were marathon runners, tri-athletes and long-distance cyclists and this calculation shows a marked inconsistency between the data provided by N balance and whole-body isotope infusion[31].

Time for a new approach?

Problems inherent to N balance and the fact that whole-body stable isotope tracers provide contradictory

calculations indicate that there are methodological problem(s). N balance has provided unreasonable estimates and attests to the warnings by many that this procedure is problematic[1–3]. Stable isotope experiments have been short term and cannot assess long-term adaptations or amino acid or protein balance throughout a daily feeding–fasting cycle. This suggests that a new experimental model or laboratory procedure may be necessary to provide an accurate picture of protein health in the habitually active individual[3,20]. Any new model must account for the various metabolic adaptations that occur with training such as: alterations in dietary protein efficiency, changes in the oxidative pathway of individual amino acids and changes in the intracellular reutilisation of amino acids from proteolytic pathways. An adaptive metabolic demand model as proposed by Millward[20] may be useful to consider in this regard (see Fig. 3). Although this model is much more labour intensive, it has the potential to allow for an accurate assessment of the athlete's dietary protein needs. Both acute and chronic adaptations to a dietary change and/or alteration in exercise volume are incorporated into the model. As outlined in Fig. 3 it can be divided into: (1) a fixed component that meets obligatory demand; (2) a variable adaptive component that is relatively insensitive to short-term dietary manipulations and that can change over the long term; and (3) inter-individual (and training-induced) variations in dietary protein efficiency. In the athlete there are losses of amino acids and protein during the fasting state and with exercise, but there are fed-state gains. Amino acids may be used to fuel exercise energy needs
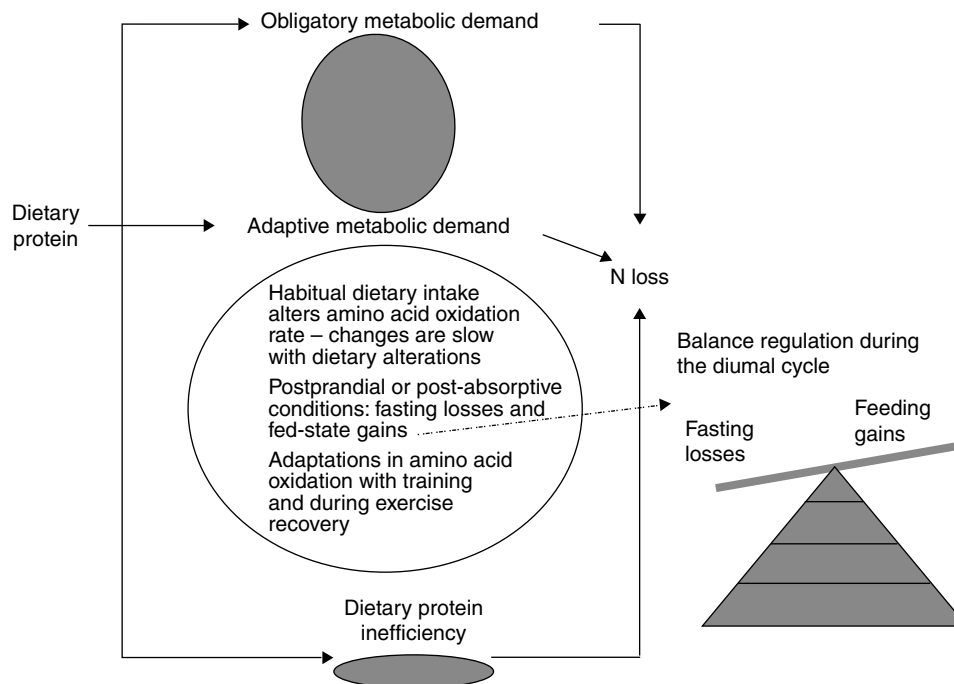

Fig. 3. Fate of dietary protein: an adaptive metabolic demand model of protein requirements as proposed by Millward[20]. This figure was altered with written permission to include those factors relevant to the sport nutrition setting. Note the addition of acute exercise and recovery effects on amino acid catabolism to the model. The adaptive behaviour of amino acid oxidation is a determinant of the metabolic demand for protein in the endurance athlete.

but the contribution is small and this process has been shown to become more efficient with habitual training. In the endurance athlete, then, protein requirements would be defined as the intake needed to balance N losses (amino acids used during exercise and recovery). Lastly, a long-term study design needs to be employed to adequately account for feeding-state gains that replenish exercise losses. Other approaches maybe useful as well. Genomic and proteonomic studies may provide a new approach to studying this issue. But until agreement can be found between laboratory procedures it seems unwise to recommend a change in dietary protein intake for the habitually active individual.

Conclusions

There are public health consequences to recommending an increase in dietary protein consumption. Even moderate changes in dietary protein have metabolic consequences[71], not the least of which are alterations in the risk for chronic disease as well as negative impacts on cancer-protective metabolites in the colon[71–73]. In addition, many unsuspecting athletes are using amino acid supplements, protein powders and pills, and run the risk of ingesting contaminants in these unregulated products. Unnecessary amino acid supplementation can induce imbalances in amino acid ingestion that may cause adverse effects[74,75]. The laboratory techniques used to justify increasing the protein RDA has had numerous experimental limitations and therefore its accuracy is difficult to ensure. The 'black-box' nature of the balance model does not allow for the assessment of those metabolic adaptations and dietary efficiencies that accrue with chronic training. Short-term studies of amino acid oxidation during exercise cannot adequately account for fed-state gains over a 24 h diurnal period. These scientific shortcomings suggest that calls to increase the protein RDA for the habitually active or endurance athlete are not substantiated. A modification in laboratory procedures and methodology may provide greater fidelity to the dietary protein requirement for the habitually active individual.

Acknowledgements

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