Protein and amino acids for athletes

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The main determinants of an athlete's protein needs are their training regime and habitual nutrient intake. Most athletes ingest sufficient protein in their habitual diet. Additional protein will confer only a minimal, albeit arguably important, additional advantage. Given sufficient energy intake, lean body mass can be maintained within a wide range of protein intakes. Since there is limited evidence for harmful effects of a high protein intake and there is a metabolic rationale for the efficacy of an increase in protein, if muscle hypertrophy is the goal, a higher protein intake within the context of an athlete's overall dietary requirements may be beneficial. However, there are few convincing outcome data to indicate that the ingestion of a high amount of protein ($2-3 \text{ g} \cdot \text{kg}^{-1}$ BW $\cdot \text{day}^{-1}$, where BW = body weight) is necessary. Current literature suggests that it may be too simplistic to rely on recommendations of a particular amount of protein per day. Acute studies suggest that for any given amount of protein, the metabolic response is dependent on other factors, including the timing of ingestion in relation to exercise and/or other nutrients, the composition of ingested amino acids and the type of protein.

Keywords: amino acids, hypertrophy, muscle, protein, strength, training.

Introduction

The importance of protein to athletes has long been recognized. From coaches of Olympians in ancient Greece to today's multi-millionaire athletes, protein has been considered a key nutritional component for athletic success. For an equally long time, the nature of that importance has been controversial. Among many athletes, especially strength and team sport athletes, protein and amino acid ingestion is considered essential to performance. Amino acid and protein supplements have become a billion dollar industry. However, information stemming from research into the efficacy of elevated protein and amino acid ingestion, as well as the requirements for top sport athletes, is still relatively sparse. There is much concerning protein nutrition for athletes that has yet to be resolved.

In 1991, Peter Lemon presented a thorough review of the state of the knowledge at that time. He reported that there was considerable controversy about the protein requirements of athletes, which, unfortunately, has yet to be resolved. Studies suggested that amino acid oxidation was increased during exercise, at least with dynamic exercise (Lemon, 1991). Thus, it was felt that habitual exercise of sufficient intensity and duration

would increase dietary protein requirements. Nitrogen balance and other studies supported this contention and recommendations for protein intake were made. Strength or speed athletes were recommended to consume about 1.2–1.7 g protein kg^{-1} BW day^{-1} and endurance athletes about 1.2-1.4 g protein \cdot kg⁻¹ $BW \cdot day^{-1}$ (where BW = body weight). Whereas these recommendations exceeded the US recommended daily allowance (RDA) for protein (0.8 g protein \cdot kg⁻¹ $BW \cdot day^{-1}$), they did not exceed the habitual dietary intake of most athletes, so it was felt that there was no reason to recommend that most athletes should increase the protein in their diet (Lemon, 1991). The main aim of the present review is to update the literature since 1991 and to critically examine the available information on protein nutrition for athletes.

Over the last 10–15 years, much has been published on the interaction of exercise and nutrition. Tissue metabolism, especially muscle, has received increasing attention as methods have been developed to examine the response of muscle protein metabolism to exercise and nutrition. Despite these advances, much remains to be resolved and many questions remain to be answered.

Technical difficulties clearly contribute to some of the confusion and disagreements surrounding protein requirements for active individuals. The selection of participants, their adaptation to the protein intake during a study, their training state, exercise intensity

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and energy balance, may all contribute to discrepant results (Butterfield, 1987; Millward, 1999). Nitrogen balance is the most common method used to estimate protein requirements. The limitations of nitrogen balance measures are well known. (Readers are referred to one of the many excellent reviews on this topic for more detailed information: Hegsted, 1976, 1978; Young, 1986; Lemon and Proctor, 1991; Tome and Bos, 2000.) However, from a practical standpoint, coaches and athletes are not usually interested in the myriad of scientific arguments for the impact of exercise on protein requirements. They simply wish to know whether a particular athlete's performance will be enhanced by consuming more, or less, protein. None of the methods that have been utilized in studies seeking to determine protein intakes for athletes has a direct relationship with athletic performance. The impact of diet on performance is what we are interested in, but it is difficult to quantify. At this time, there is no clear consensus on the importance of elevated protein for the athletic population.

Meanwhile, there is, perhaps, a more fundamental problem for determination of the protein requirements of athletes. Much of the controversy surrounding protein requirement in athletes may be due to the exact meaning of this term for this population. In our opinion, it is difficult to define protein requirement as it applies to competitive athletes. The most commonly used definition for the nutritional requirement for protein is the minimum amount ingested that will balance all nitrogen losses and thus maintain nitrogen equilibrium (Millward, 2001). Losses are considered to be the obligatory nitrogen losses (i.e. all nitrogen losses on a protein-free diet), thus representing the minimum metabolic demands for amino acid nitrogen. This definition is based on nitrogen balance studies and it, or something very similar, has typically been used in studies that examined the impact of exercise on protein requirements (Tarnopolsky et al., 1988, 1992; Meredith et al., 1989; Lemon et al., 1997; Forslund et al., 1998). Millward (2001) also suggested a more complex definition of protein requirement based on the Adaptive Demands Model, in which the metabolic demands include both obligatory and adaptive components. Thus, protein requirement is defined as the minimum protein intake that satisfies the metabolic demands and which maintains body composition. For the sedentary population, the US RDA for protein is 0.8 g protein kg^{-1} BW day^{-1} , but it is not clear whether athletes in training need more than this amount.

These definitions may be appropriate for normal, healthy or even clinical populations, but we feel that neither is necessarily appropriate for athletes in training. Accepted definitions of protein requirement are based on nitrogen balance measures in the laboratory and may have no relationship with athletic performance. Athletes and coaches are more interested in the optimum protein intake for athletic success, rather than the actual definition of protein requirement based on nitrogen balance. In fact, the appropriate definition of protein requirement - that is, the optimal protein intake - will vary depending on the training and competition goals of the athlete. It is important to bear in mind the goals of different athletes when discussing the importance of protein ingestion. For each individual, the impact of increased protein on whole-body and muscle nitrogen balance must be considered in terms of the different nitrogen metabolic pathways (Tome and Bos, 2000). For example, an endurance athlete would probably consider the protein necessary to maintain lean body mass and not impair performance as the protein requirement. Aside from obligatory uses, protein would be necessary for increased energy demands, protein synthesis of enzymes that are stimulated by endurance training and, perhaps, repair of muscle proteins damaged by intense training. On the other hand, a strength athlete and many team sport athletes, such as rugby or hockey players, would not consider the intake necessary for maintenance of lean body mass sufficient, but rather the protein requirement would be the amount necessary to increase muscle mass and strength or power. Additionally, not only would the definition be different for athletes in different sports, but also in individual circumstances (e.g. weight loss) in any given sport. For athletes, muscle protein is possibly the most important body protein to consider. Those athletes desiring muscle hypertrophy expect their training and diet to stimulate protein accretion. Clearly, chronic exercise training has a dramatic impact on muscle protein metabolism. Exercise training results in fundamental adaptations in the muscle. The metabolic basis for protein accretion is net muscle protein synthesis that is, protein synthesis exceeds protein breakdown over the given period in question. The primary adaptation to resistance exercise training is muscle hypertrophy, whereas endurance training results in increased muscle oxidative capacity. The response of muscle protein metabolism to the training stimulus has a major impact on these adaptations. Very little research has examined the chronic response of muscle protein metabolism to exercise training. Phillips et al. (1999) demonstrated that the responses of mixed-muscle (i.e. the weighted average of all proteins in muscle) protein synthesis and breakdown to acute resistance exercise were ameliorated in resistance-trained individuals compared with untrained individuals (Fig. 1). These and more recent results (Phillips et al., 2002) are consistent with the principle of general adaptation to training. That is, as a system - in this case, muscle adapts to a stress, greater stress must be added to

generate the desired adaptations. Muscle mass clearly adapts to the type and severity of training, as well as the nutritional intake of the athlete. To maintain muscle mass at greater than 'normal' (i.e. that which is genetically determined), muscle protein synthesis must be increased regularly. Over 24 h, the response of muscle to resistance exercise plus hyperaminoacidaemia is additive to the normal 24-h muscle protein balance (Tipton et al., 2003) (Fig. 2). To date, there have been no studies on the impact of chronic endurance training on muscle protein metabolism. However, endurance training increases the activity and amount of mitochondrial enzymes and the size and amount of muscle mitochondria (Neufer, 1989), suggesting that muscle protein metabolism, at least for some proteins, must be influenced by endurance training.

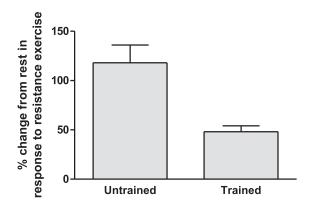


Fig. 1. Change in mixed-muscle fractional synthetic rate in response to a resistance exercise bout in untrained and resistance-trained individuals (from Phillips *et al.*, 1999).

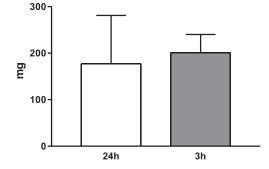


Fig. 2. Difference in phenylalanine exchange (area under the curve for net muscle protein balance in mg) between rest and exercise + essential amino acid supplement (ES) over the entire 24 h and over 3 h, in both trials, corresponding to the time of amino acid ingestion and exercise in the ES trial. Thus, the response to exercise and amino acid ingestion for 3 h represents the response over the entire 24-h period (from Tipton *et al.*, 2003).

Interestingly, both endurance and resistance exercise increase mixed-muscle protein synthesis. Clearly, muscular adaptation to endurance exercise is profoundly different from that to resistance exercise. Thus, it is difficult to accept that the response of muscle protein metabolism to an acute bout is the same for both types of training. There are several possible explanations for why the response of mixed-muscle protein synthesis to endurance and to resistance exercise is qualitatively similar. Mixed-muscle protein synthesis represents the weighted average of all the proteins in the muscle, each of which may have a unique response to a particular type of exercise. Certainly, since the major result of chronic resistance training is muscle hypertrophy, it is intuitive to expect resistance exercise to influence myofibrillar more than mitochondrial proteins; the converse would be true for endurance exercise (Table 1). In the past, methodological limitations prevented the detailed examination of the response of synthesis of different protein sub-fractions to exercise. Today, however, the techniques are available to examine muscle protein sub-fraction (Welle et al., 1995; Rooyackers et al., 1997; Bohe et al., 2001) and even individual protein synthesis (Balagopal et al., 1994; Hasten et al., 2000). These methods indicate that myofibrillar protein synthesis responds to resistance exercise in a similar manner to mixed-muscle protein synthesis (Balagopal et al., 1997a,b, 2001; Hasten et al., 2000). Unfortunately, to date, no study has compared the response of synthesis of different types of proteins to different forms of exercise. Moreover, there is no means to directly measure degradation of different protein sub-fractions or individual proteins in humans in vivo. Determination of the net balance of protein subfractions is not possible.

There remains much controversy about the protein requirement of athletes. The following discussion will make it apparent that the results of studies appear to conflict. There is evidence to support the argument for both the efficacy of increased protein intake (i.e. intake

 Table 1. Measured and proposed changes of mixed-muscle,

 myofibrillar and mitochondrial protein synthesis in response to

 different types of exercise

| | Endurance exercise | Resistance exercise |
|------------------------------------|-------------------------------------|-----------------------------------|
| Mixed-muscle protein synthesis | ↑ | \uparrow or $\uparrow\uparrow$ |
| Myofibrillar protein synthesis | \leftrightarrow or \downarrow ? | \uparrow or $\uparrow\uparrow$ |
| Mitochondrial protein synthesis | ↑ | \leftrightarrow or \uparrow ? |

greater than the RDA for sedentary individuals) as well as for protein intakes that are similar to those of sedentary individuals. So, we will attempt to illustrate the data supporting arguments both for and against higher intakes for top sport athletes. Moreover, we hope to convey the notion that a general value for protein requirements for athletes is difficult to ascertain and determination of appropriate protein intake must take into account the demands of the individual goals and training regimen of each athlete.

Protein requirements

Since the original consensus report in 1991, protein requirements have been examined in additional studies, but controversy continues regarding the impact of exercise, especially exercise as intense as that top sport athletes must perform, on protein requirements (Tarnopolsky, 1999; Rennie and Tipton, 2000; Wolfe, 2000). The basis of the argument for increased protein needs by exercising individuals is often an increase in amino acid (usually represented by leucine) oxidation during dynamic exercise. Leucine oxidation was found to be increased during dynamic exercise in recent studies (Tarnopolsky et al., 1992; Phillips et al., 1993; El Khoury et al., 1997; Lemon et al., 1997; Bowtell et al., 1998, 2000; Forslund et al., 1998; Lamont et al., 1999; Millward, 1999). Increased amino acid oxidation during exercise is thought to be due to increased utilization of amino acids as fuel; therefore, regular and repeated exercise would then lead to increased protein requirements.

On the other hand, previous work from our laboratory showed that the fate of leucine during exercise might not reflect that of all amino acids and leucine oxidation is not matched by urea production (Wolfe et al., 1984). Also, lysine oxidation was not increased, suggesting that short-term increases in leucine oxidation do not reflect whole-body protein breakdown. Training does not increase branched-chain amino acids (BCAA) oxidation (Lamont et al., 1999) and, in fact, may attenuate the increase due to exercise (McKenzie et al., 2000). Furthermore, increased amino acid oxidation during exercise must influence 24-h oxidation of amino acids and thus nitrogen balance for protein requirements to be changed. The 24-h leucine oxidation and balance were measured in healthy men performing 180 min of cycling exercise at protein intakes of 1.0 and 2.5 g protein \cdot kg⁻¹ BW \cdot day⁻¹ (El Khoury et al., 1997; Forslund et al., 1998). Whereas leucine oxidation was increased during exercise and by the higher protein intake, 24-h whole-body leucine remained in equilibrium at both protein intakes. It is thought that the increased leucine oxidation during

exercise was compensated for by small and undetectable changes throughout the day. Support for this notion comes from Devlin et al. (1990), who showed that during 3 h of cycling at 75% $\dot{V}O_{2max}$, leucine oxidation was less than at rest. These data do not support the contention that dynamic exercise increases protein requirements. The impact of resistance exercise on amino acid oxidation has received far less attention, but there is no indication that resistance exercise increases leucine oxidation either during or after exercise (Tarnopolsky et al., 1991). Thus, it seems that the whole-body amino acid data are somewhat equivocal and it cannot be concluded with certainty that habitual exercise increases protein requirements. Leucine oxidation appears to be increased by endurance exercise, but this increase may not reflect an increased requirement for dietary protein.

Nitrogen balance is the most common method used to determine protein requirements. Using this method, many authors have concluded that both endurance and resistance athletes require more than the 0.8 g protein \cdot kg⁻¹ BW \cdot day⁻¹ dietary recommendation (Lemon, 1991). The results of more recent studies support this argument (Tarnopolsky et al., 1992; Phillips et al., 1993). Feeding athletes different amounts of protein and measuring nitrogen balance, then extrapolating to the zero line, was the basis for the conclusion that athletes require more dietary protein. In all these studies, zero balance was calculated to be above 0.8 g protein \cdot kg⁻¹ $BW \cdot day^{-1}$; thus, a widely accepted notion that athletes need more protein has been promulgated. It is important to note that nitrogen balance is the method that is commonly used to determine the protein needs of sedentary populations; thus comparing like to like, these studies indicate that active individuals, particularly athletes, require more protein in their diet.

On the other hand, the notion that athletes require more protein than sedentary individuals is not universally accepted. There is ample evidence to suggest that protein needs are not increased by habitual exercise. In fact, the opposite interpretation - that is, exercise training increases the efficiency of protein utilization, thus making increased intake unnecessary has been argued. In a series of studies from the laboratory of the late Gail Butterfield, it was demonstrated that protein utilization is increased and protein requirements are decreased by endurance exercise (Butterfield and Calloway, 1984; Todd et al., 1984). Similarly, in a classic study, Gontzea et al. (1975) demonstrated that nitrogen balance was initially negative upon initiation of an exercise training programme while maintaining constant protein intake, but returned to equilibrium after a period of accommodation. Following the period of accommodation, no increase in protein intake was necessary to maintain nitrogen equilibrium. These studies suggest that it is not necessary for physically active individuals to increase protein intake to maintain nitrogen equilibrium; in fact, exercise may decrease protein needs due to increased efficiency of protein utilization. Critics of this line of thought suggest that the intensity and volume of training in the aforementioned studies was not equivalent to that of top sport athletes and thus may not represent the circumstances the elite athlete faces (Lemon, 1991; Lemon and Proctor, 1991).

A similar argument can be made for strength athletes. Acute resistance exercise in the fasted state has been shown to improve net muscle protein balance (Biolo *et al.*, 1995b; Phillips *et al.*, 1997). Furthermore, training ameliorates the response of muscle to a bout of resistance exercise in both the fasted (Phillips *et al.*, 1999) and fed (Phillips *et al.*, 2002) states. These data suggest that there is an accommodation of muscle protein metabolism such that protein needs for maintenance of body mass in trained individuals would not be increased. On the other hand, maintenance of lean body mass is not the goal of many athletes, especially those who participate in strength and power sports, and so the protein needed to increase body mass might be greater.

Alternative interpretations of the nitrogen balance data can be used to argue that strength athletes may want to consume large amounts of protein. If an athlete is trying to increase muscle mass, then the goal would not be nitrogen equilibrium, but positive nitrogen balance. Examination of the nitrogen balance data presented by Tarnopolsky et al. (1992) for strengthtrained athletes could be used to support the contention that increased protein intake will increase nitrogen balance in athletes during intense resistance training. There are data to support the notion that all other things being equal, the more protein ingested, the more lean body mass may be increased. Indeed, Hegsted (1978) summarized a variety of nitrogen balance studies of adults, children and pregnant women. Taken together, these studies indicate that there is a retention of $\sim 20\%$ of the intake above the maintenance need, at least at intakes above maintenance (~0.5 g nitrogen kg^{-1} BW day^{-1}). Furthermore, this retention appeared to continue for as long as the studies were conducted. Tome and Bos (2000) summarize studies that indicate high protein intakes can result in continuous, positive nitrogen balance from 1 to 3 g nitrogen day^{-1} . For example, positive nitrogen balance was maintained for up to 50 days on a very high protein diet (\sim 3 times the RDA) with no adaptation evident (Oddoye and Margen, 1979). To date, there are no nitrogen balance studies that show anything other than increased positive nitrogen balance when protein intake is increased. Interpreted this way, it could be

argued that athletes who desire muscle hypertrophy should eat very high protein diets to maximize their muscle gain. These data illustrate that high, positive nitrogen balance can be maintained for an extended period (Oddove and Margen, 1979), thus providing the capacity for muscle hypertrophy. Whole-body (Forslund et al., 1998) and muscle (Borsheim et al., 2002; Miller et al., 2003) protein synthesis rates are increased with increasing protein or amino acid intake, supporting the notion that the specific requirement for protein intake may be determined by the desired lean body mass. Furthermore, there is evidence from one study that the increase in lean body mass during resistance exercise training is greater with increased protein intake (Burke et al., 2001). Weightlifters were fed either 1.2 or 2.1 g protein \cdot kg⁻¹ BW \cdot day⁻¹. The group that ingested the higher amount of protein had increased lean body mass compared with the group receiving less protein (Fig. 3). Results from a recent bed rest study from our laboratory further support the notion that increased nitrogen intake may increase muscle mass. Two groups of individuals rested in bed for 28 days (Paddon-Jones et al., 2003). Both groups were fed weight maintenance diets, but one was given additional nitrogen in the form of an essential amino acid supplement. Whereas the placebo group lost muscle mass, the supplemented group maintained muscle mass during the bed rest period. Furthermore, although both groups lost strength, the loss was significantly ameliorated by the increase in nitrogen.

Not all available evidence supports the notion that ingesting greater amounts of protein leads to greater lean body or muscle mass. The important result of dietary protein intake is maintenance – or increase, in the case of strength and power athletes – of lean body mass. Interestingly, if calculations of body composition

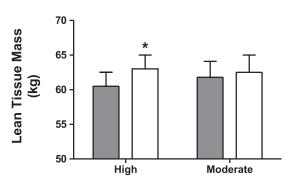


Fig. 3. Change in lean body mass in experienced weightlifters consuming either high $(2.1 \text{ g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1})$ or moderate $(1.2 \text{ g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1})$ dietary protein during a 6-week resistance training programme. \blacksquare , before training; \square , after training. *After training significantly different from before training (adapted from Burke *et al.*, 2001).

are made from the apparent nitrogen retention at high protein intakes, unrealistic estimates of body composition can result (Hegsted, 1976, 1978). Furthermore, studies in which lean body mass was measured during resistance exercise training while consuming different amounts of protein do not offer a clear-cut conclusion that protein needs are greater in the athletic population. That is, nitrogen balance may indicate high nitrogen retention with increased protein intake without any increase in muscle mass (Tarnopolsky et al., 1988, 1992). However, these studies were short (on the order of a few weeks), so it is possible that protein accretion was occurring, but was not sufficient to be measured with existing body composition methods. In a 12-week study, elderly individuals were fed the RDA (0.8 g protein \cdot kg⁻¹ BW \cdot day⁻¹) or double the RDA for protein during a resistance-training programme (Campbell et al., 1995). Both nitrogen retention and body composition were similar in the two dietary protein groups. Of course, it is possible that young athletes would have an entirely different response, so the relevance of this study to the athletic population may be questioned. Three groups of rats were fed different amounts of protein during functional overload to stimulate muscle hypertrophy in the overloaded muscle (O'Hagan et al., 1995). Protein intake was 7, 17.5 or 30% of the diet in the three groups. The amount of dietary protein did not affect the experimentally induced muscle growth in either the plantaris or soleus muscles. Thus, it is not clear whether the increased nitrogen retention associated with increased protein intake leads to greater lean body mass or muscle hypertrophy.

If nitrogen retention increases, but does not result in increased lean body mass and muscle mass, then what is the fate of the excess nitrogen? Certainly, whole-body amino acid oxidation increases with increased protein intake (Tarnopolsky et al., 1992; Bowtell et al., 1998; Forslund et al., 1998), but the nitrogen must end up somewhere. It is possible that the amount of protein deposition is too small to be detected by body composition methodology. Alternatively, excretion of nitrogen could be underestimated; thus the apparent nitrogen retention may be due to inherent errors in the calculation of nitrogen balance. Exercise increases urea nitrogen recycling into body proteins (Carraro et al., 1993). Furthermore, increased protein breakdown appears to provide amino acids for plasma proteins (Carraro et al., 1990). However, how this relates to long-term changes in nitrogen balance is unclear. Thus, it is uncertain how these factors would be accounted for in calculations of the fat-free mass from nitrogen retention.

In any discussion of protein requirements, the importance of energy intake must be considered. There

is evidence to suggest that energy intake is, in fact, more important for maintenance of nitrogen equilibrium than protein intake. Butterfield and co-workers clearly demonstrated the importance of energy for maintenance of nitrogen balance and lean body mass (Todd et al., 1984; Butterfield, 1987; Butterfield et al., 1997). Almost 100 years ago, it was demonstrated that athletes can gain strength and maintain mass on relatively small protein intakes, as long as sufficient energy is available (Chittenden, 1907). Furthermore, energy intake may be crucial for nitrogen retention and increased lean body mass. In the study of Gater et al. (1992), individuals were resistance trained for 10 weeks on one of three dietary regimens. One group performed the training with no additions to their diet, a second was given an amino acid supplement and a third consumed a diet with positive energy balance. Positive energy balance without additional protein engendered the greatest gains in lean body mass during the 10-week training programme. Other investigators also have demonstrated that increases in lean body mass result from increased energy intake in sedentary individuals (Forbes et al., 1986, 1989; Welle et al., 1989; Jebb et al., 1993). So, it would appear that increased energy intake may be crucial to increasing lean body mass during training, possibly more so than protein intake.

Even if we accept that the protein needs of athletes are greater than the RDA, it should not necessarily be a reason for the broad recommendation that athletes increase their protein intake. Most athletes, especially those with training volumes such that energy intake is necessarily high, probably consume sufficient protein in their normal diet (Deuster et al., 1986; Grandjean, 1989), even those from underdeveloped countries (Christensen et al., 2002), to cover even the higher recommendations that have been published (Lemon, 1991; Lemon and Proctor, 1991). Viewed another way, even athletes whose muscle hypertrophy is maximized are probably eating sufficient protein to cover the need for muscle protein accretion (Millward et al., 1994). Millward et al. (1994) point out that even the rates of steroid-induced muscle growth are trivial ($\sim 3\%$) relative to normal dietary intake. Furthermore, the increased energy demands of intense resistance training would increase dietary protein intake such that the diet would supply at least 50% more protein than the maximum rate of accretion that has been reported. Thus, for most athletes, recommendations for increased protein do not seem necessary. Some athletes who need to control their weight may need to increase protein as a proportion of their energy intake. Nitrogen balance is better maintained on a hypoenergetic diet if protein intake is high. Furthermore, as discussed in more detail below, examination of the available data has led to the conclusion that athletes wishing to increase muscle mass

may want to consume large amounts of protein. For example, the argument that strength athletes consuming sufficient energy will consume more than enough protein to supply amino acids for protein accretion (Millward *et al.*, 1994) presumes that there is a direct relationship between protein intake and protein accretion. There is ample evidence that amino acids not only function as precursors for protein synthesis, but also act as regulatory molecules to stimulate net muscle protein synthesis (Wolfe and Miller, 1999; Kimball, 2002; Kimball and Jefferson, 2002); thus that there is a direct relationship between protein intake and protein accretion is not necessarily correct.

In addition to limitations of nitrogen balance studies, the discrepancies may be rooted in the inherent limitations of long-term endpoint studies. Whereas longitudinal endpoint studies might best be utilized to answer the question of whether or not increased dietary protein increases muscle mass, these studies are difficult to perform with sufficient control to render readily interpretable results. Strict control of all aspects of an athlete's life (e.g. training, rest, sleep, diet, travel) for a lengthy period is necessary to obtain measurable results. The problem is that these studies may not demonstrate efficacy of increased protein intake even if effective. Assuming that increasing protein intake is effective for increasing muscle mass, the response is likely to be small compared with the normal response to exercise and meal intake, especially for elite athletes. The major stimulus for muscle growth will be the training regimen and normal nutrient intake. Any additional stimulus due to elevated protein intake would be relatively minor. On the other hand, a small response that is difficult to measure may, in fact, be physiologically relevant and, if so, increased protein intake would be important to a nutritional programme. Difficulties with control of variables in longitudinal studies contribute to the uncertainty and it may be virtually impossible to conduct a study with appropriate control that will demonstrate clearly that increased protein results in increased lean body mass.

Thus far, the discussion of protein requirements has strictly been in the context of the amount of protein necessary in the diet. There is abundant evidence to suggest that determination of protein needs is not as simple as merely expressing a quantity to be ingested per day. Evidence from acute metabolic studies, as well as longitudinal studies, indicates that other factors, such as composition of the protein and amino acids, timing of ingestion and other nutrients ingested concurrently, influence the utilization of ingested protein and amino acids. Thus, for any given protein intake, factors important to an athlete's performance may vary depending on exactly what is ingested and when it is ingested.

Composition of ingested protein and amino acids

Amino acid composition appears to influence the response of muscle protein balance following resistance exercise. Net muscle protein synthesis results from ingestion of essential amino acids only (Tipton et al., 2001, 2003; Borsheim et al., 2002). Thus, it is clear that non-essential amino acids are not necessary for the stimulation of muscle protein synthesis resulting in net muscle protein synthesis. There is evidence that even single amino acids may stimulate protein synthesis and possibly net muscle protein synthesis. Studies in rats demonstrate that leucine ingested after running results in increased muscle protein synthesis by stimulating the protein translation initiation pathways (Gautsch et al., 1998; Anthony et al., 1999). In resting humans, muscle protein synthesis was stimulated by bolus doses of single essential, but not non-essential, amino acids (Smith et al., 1994). Thus, individual amino acids may act as stimuli for muscle protein synthesis and positive net muscle protein balance. These results suggest that essential amino acids may act to stimulate muscle protein synthesis in two ways: (1) by supplying substrate for muscle protein synthesis and (2) acting as a regulatory factor.

The amount of essential amino acids necessary to acutely stimulate muscle protein synthesis and net muscle protein synthesis appears to be relatively small. Ingestion of as little as 6 g of essential amino acids, both with (Tipton et al., 2001) and without carbohydrates (Borsheim et al., 2002), results in dramatic elevations in muscle protein synthesis leading to net muscle protein synthesis. Moreover, some data suggest that there is a dose dependency of the response of muscle protein synthesis to essential amino acid ingestion after exercise. The response of net muscle protein balance to two doses of 6 g each of essential amino acids (Borsheim et al., 2002) was about double that of two doses of 6 g each of mixed amino acids (Miller et al., 2003). The mixed amino acids contained approximately 3 g of essential amino acids in each dose or about half the amount in the two 6-g doses. Thus, there may be a point of essential amino acid availability above which no further stimulation occurs. Support for this concept comes from the fact that net muscle protein synthesis was similar when ~ 20 g and 40 g of essential amino acids were ingested after resistance exercise (Tipton et al., 1999). Any further stimulation must occur following the next meal. A summary of the response of net muscle protein balance to amino acids, as well as carbohydrates (discussed below), is illustrated in Fig. 4. At this time, the minimum dose of essential amino acids necessary to stimulate net muscle protein synthesis and the amount necessary to stimulate the

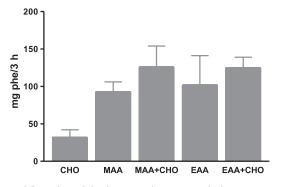


Fig. 4. Net phenylalanine uptake over 3 h in response to carbohydrate and amino acid ingestion after resistance exercise in healthy volunteers. CHO = 100 g of sucrose in a single bolus. MAA = 2×6 g of mixed – that is, non-essential and essential amino acids (6 g of essential amino acids total) – ingested at 1 and 2 h post-exercise. MAA+CHO = 2×6 g of mixed amino acids (6 g of essential amino acids total) + 35 g of sucrose ingested at 1 and 2 h post-exercise. EAA = 2×6 g of essential amino acids total) only ingested at 1 and 2 h post-exercise. EAA+CHO = 6 g of essential amino acids (6 g of essential amino acids total) + 35 g of sucrose ingested at 1 and 2 h post-exercise. EAA+CHO = 6 g of essential amino acids (6 g of essential amino acids total) + 35 g of sucrose ingested at 1 h post-exercise.

maximum response remain to be determined. Further studies must be conducted to determine the upper level of this dose dependency of muscle protein balance to essential amino acid intake. The dose–response relationship may help explain why further increases in protein intake will not result in chronic increases in muscle mass.

The response of muscle protein synthesis and net muscle protein balance to hyperaminoacidaemia may be linked to intracellular amino acid availability (Wolfe and Miller, 1999; Wolfe, 2000). Ingestion of a nutrient that results in hyperaminoacidaemia increases amino acid delivery to the muscle, transport into the muscle cell and intracellular amino acid availability. Increased blood flow due to the exercise bout, as well as elevated rates of protein synthesis due to the exercise, would lead to increased amino acid delivery to the muscle and the potential for increased muscle protein synthesis after exercise. Taken together, these factors may explain the additive effect of exercise and amino acids on net muscle protein balance (Biolo et al., 1997). Alternatively, or in addition to, increased intracellular availability of amino acids, there is evidence linking the stimulation of muscle protein synthesis to the change in arterial amino acid concentrations (Borsheim et al., 2002). Borsheim et al. (2002) showed that arterial essential amino acids increased several-fold in response to ingestion of 6 g of essential amino acids after resistance exercise. Net muscle protein balance increased proportionally more than arterial concentra-

tions and declined rapidly when arterial amino acid concentrations began to decline. Interestingly, net muscle protein balance had returned to resting values when arterial amino acid concentrations were still double resting values. Muscle intracellular amino acid concentrations increased, but were not different from resting values by the end of the study (Borsheim et al., 2002). These results are similar to those of a previous study demonstrating rapid changes in net balance without lasting changes in muscle intracellular concentrations (Tipton et al., 2001). Moreover, another study from our laboratory demonstrated that decreased blood amino acid concentrations resulted in decreased muscle protein synthesis and restoration of arterial amino acids resulted in restoration of synthesis (Kobayashi et al., 2003). Much of these changes can be explained by the notion that arterial amino acid concentrations are the key regulating factor. It is possible that muscle protein may be playing a central role in maintenance of blood amino acids. Thus, the changes in muscle protein breakdown and muscle protein synthesis are responsive to the concentration of essential amino acids in the blood and net muscle protein synthesis occurs only when there is an increase in arterial concentrations. In this scenario, the changes in muscle protein synthesis and breakdown may provide amino acids for other tissues (e.g. liver proteins; Wolfe et al., 1984; Carraro et al., 1990).

On the one hand, transiently changing intracellular amino acid concentrations would not appear to support the notion that intracellular amino acid availability regulates muscle protein synthesis and net muscle protein balance (Gibala, 2001). However, the intracellular amino acid pool and rates of metabolic processes are in a constant state of flux. Stimulation of muscle protein synthesis would increase utilization of the amino acids coming into the cell from increased transport, as well as muscle protein breakdown. Any elevation of muscle intracellular amino acid concentrations would thus be transient and may not be measured unless muscle was sampled on a frequent, perhaps even minute-by-minute, basis. It would appear that the two notions of control of muscle protein synthesis are not mutually exclusive. Therefore, the regulation of muscle protein synthesis and net muscle protein balance by amino acids following exercise may respond to either changes in concentrations of arterial amino acids or intracellular amino acid availability, or both.

It is not necessary for hyperaminoacidaemia to result from ingestion of only free amino acids. On a wholebody level, recent studies have demonstrated that a response characterized by a prolonged amino acid appearance of lesser magnitude is superior to a response characterized by a more transient appearance of greater magnitude (Boirie *et al.*, 1997; Dangin *et al.*, 2001). Ingestion of whey proteins resulted in amino acid concentrations in the blood that were higher and less prolonged than those resulting from casein (Boirie et al., 1997). Both resulted in positive whole-body protein balance, but the anabolic response to casein was superior to that of whey proteins. The digestive properties for these proteins are different such that amino acids from casein appear more slowly than those from whey proteins (Dangin et al., 2001). The digestive properties of the proteins appear to play a role in the anabolic response to ingestion on a whole-body level, but it is unclear if the anabolic response of muscle is similar or if exercise influences this response. Thus, whereas it is clear that supply of amino acids is the critical factor for stimulating net muscle protein synthesis following resistance exercise, other factors may also play a role.

Net positive muscle protein synthesis clearly results when amino acids are ingested after resistance exercise, but the response after endurance exercise is not as clear. Blomstrand and Saltin (2001) fed participants a BCAA solution during and after cycling exercise. Ingestion of the solution resulted in slight improvements in net muscle protein balance, but the balance remained negative - that is, net muscle protein synthesis did not result. These results could be interpreted to suggest that the response to endurance exercise combined with amino acid intake is different from that to resistance exercise. Alternatively, they could be interpreted to suggest that feeding BCAA only is not sufficient to stimulate net muscle protein synthesis after exercise. This notion is supported by the fact that net uptake of amino acids resulted when participants were fed a protein-carbohydrate-lipid solution after 60 min of cycling (Levenhagen et al., 2002). Thus, it is more likely that BCAA alone are insufficient to stimulate net muscle protein synthesis and that, given the appropriate nutritional stimulation, net muscle protein synthesis will occur after endurance exercise. However, since the usual response to chronic endurance training is not hypertrophy of muscle, the proteins that respond are probably different from those that respond to nutrient ingestion after resistance exercise. Studies must be conducted to determine the differences between the responses of muscle protein to endurance exercise and those to resistance exercise.

Few studies have examined the impact of carbohydrates and fats on the response of net muscle protein balance after exercise. Any effect of consuming carbohydrates or fats upon net muscle protein balance will probably primarily be due to the resulting elevation of hormones, such as insulin. Carbohydrate ingestion results in stimulation of insulin release. Local insulin infusion, such that arterial amino acid concentrations are maintained, has been demonstrated to stimulate muscle protein synthesis at rest (Biolo et al., 1995a). However, after resistance exercise, additional stimulation of muscle protein synthesis over and above that due to the exercise was limited (Biolo et al., 1999). Whereas ingestion of carbohydrates alone after resistance exercise improves muscle protein balance, without amino acids present the balance does not reach positive levels (Miller et al., 2003). Thus, it would appear that while the response of muscle protein synthesis to exercise and amino acids is additive, the response to exercise and insulin is not additive. The normal post-exercise stimulation of muscle protein breakdown, on the other hand, was ameliorated by local insulin infusion after resistance exercise (Biolo et al., 1999). The amelioration of muscle protein breakdown by insulin infusion resulted in improved net muscle protein balance over the basal, post-exercise value; however, this improvement did not result in positive balance in the absence of amino acid intake. Taken together, the decrease in muscle protein breakdown due to the insulin and the increase in muscle protein synthesis due to increased amino acids suggest that addition of an insulin secretagogue, such as carbohydrate, to an amino acid source may result in an optimal response of muscle protein balance.

No study has compared the anabolic response to the addition of lipids to amino acids to that of amino acids alone but, given the potential importance of digestive properties to the response, it should not be surprising if there was some effect. A recent study provided clear evidence, at least on a whole-body level, that ingesting proteins as part of a meal engendered a different response than when the proteins were ingested alone (Dangin *et al.*, 2003). The rate of appearance of amino acids in the blood suggests that the digestion rate of whey proteins was slower in the presence of carbohydrates and fats than without and the anabolic response was improved. Clearly, this factor could have implications for optimal protein intake for athletes.

Alternatively, the energy provided by these nutrients acutely might act to stimulate net muscle protein balance. It has been suggested that stimulation of muscle protein synthesis and the resultant net muscle protein synthesis due to nutrient ingestion is due to increased energy availability. However, in resting humans, lipid infusion was found not to result in elevated muscle protein synthesis or net balance (Svanberg *et al.*, 1999), suggesting that, at rest, increased energy per se is not an effective stimulator of muscle protein synthesis. Energy may be especially important after exercise. Since energy demand is increased after exercise, muscle protein synthesis may be more likely to be limited by energy availability, such that any elevation of muscle protein synthesis resulting in net muscle protein synthesis as a consequence of feeding is due to

replenishment of energy rather than hyperaminoacidaemia or hyperinsulinaemia per se. Support for this concept comes from in situ studies on rat muscle showing that diminished rates of protein synthesis after exercise are linked to reduced concentrations of adenosine triphosphate and phosphocreatine (i.e. a reduced energy state) (Bylund-Fellenius et al., 1984). However, in vivo studies do not support this hypothesis. Rats fed a carbohydrate-containing meal post-exercise did not demonstrate elevated rates of muscle protein synthesis; however, muscle protein synthesis was increased in rats fed an isoenergetic meal of protein plus carbohydrates (Gautsch et al., 1998). Recent studies from our laboratory demonstrate that ingestion of additional energy by human volunteers after resistance exercise does not increase in vivo net muscle protein synthesis without additional amino acids (Miller et al., 2003). After endurance exercise, ingestion of a carbohydrate-lipid supplement did not result in positive net muscle protein balance, but ingestion of carbohydrates, lipids and proteins did result in net muscle protein synthesis in human volunteers (Levenhagen et al., 2002). These results suggest that acute provision of energy per se does not have an influence on muscle protein metabolism after exercise. These results also demonstrate that addition of lipids does not increase muscle protein balance over and above that seen with protein and carbohydrates alone. However, as discussed previously, chronic energy intake has clearly been demonstrated to influence nitrogen balance (Todd et al., 1984; Butterfield, 1987), suggesting that as long as energy balance is sufficient on a chronic basis, other factors are responsible for the response of muscle protein synthesis and muscle protein balance.

Importance of timing of ingestion of nutrients in relation to exercise and other nutrients

The timing of nutrient ingestion also influences the anabolic response after exercise. In a recent study, participants ingested 6 g of essential amino acids plus 35 g of carbohydrates twice in relation to an acute resistance exercise bout (Tipton et al., 2001). The response of net muscle protein balance was considerably greater when the solution was ingested immediately before exercise than immediately after exercise. In a similar study, there was no significant difference in net muscle protein balance between trials with ingestion of the same carbohydrate-amino acid solution at 1 versus 3 h post-exercise (Rasmussen et al., 2000). Further comparison of the two studies indicated that the response of net muscle protein balance was greatest when the carbohydrate-amino acid mixture was consumed immediately before exercise. These results are

summarized in Fig. 5. It has also been demonstrated that the anabolic response to resistance exercise is influenced by the timing of ingestion of a mixture of carbohydrate, fat and protein and carbohydrates alone (Roy *et al.*, 1997, 2000). There is also support for this notion from a longitudinal study design. Elderly volunteers were given protein-containing supplements either immediately after resistance exercise or 2 h after exercise (Esmarck *et al.*, 2001) during 12 weeks of resistance training. Those receiving the supplement immediately after exercise experienced greater increases in muscle mass and strength than those receiving the supplement 2 h after exercise.

There is preliminary evidence that the timing of carbohydrate ingestion relative to amino acid ingestion may be important. After resistance exercise, amino acid uptake was greatest in the first hour following amino acid ingestion and declined during the next 2 h (Miller *et al.*, 2003). On the other hand, amino acid uptake was lowest in the first hour after ingestion of carbohydrates, but increased in the next 2 h after ingestion (Fig. 6). These results suggest that the greatest response of muscle protein balance to amino acid and carbohydrate intake may result from staggering the ingestion of carbohydrates and amino acids, such that the responses are superimposed and thus maximized. Studies need to be conducted to test this hypothesis.

After endurance exercise, it would appear that timing of nutrient ingestion also may be important for the response of net muscle protein balance. It has been shown that a protein, carbohydrate and lipid supplement resulted in greater amino acid uptake when ingested immediately after cycling exercise than 3 h after cycling (Levenhagen *et al.*, 2001). In another study, post-exercise macronutrient ingestion resulted in an attenuation of body weight loss and improved

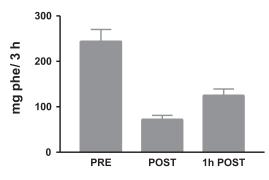


Fig. 5. Net phenylalanine uptake over 3 h after the ingestion of a solution containing 6 g of essential amino acids and 35 g of carbohydrates at three times in relation to acute resistance exercise. PRE = solution ingested immediately before exercise; POST = solution ingested immediately after exercise; 1 h POST = solution ingested 1 h after completion of exercise. Data from Tipton *et al.* (2001) and Rasmussen *et al.* (2000).

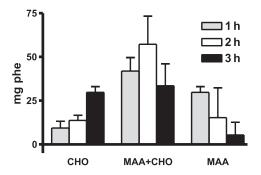


Fig. 6. Net phenylalanine uptake after ingestion of amino acids (MAA), carbohydrates (CHO) and the combination of the two (MAA+CHO) at 1, 2 and 3 h after an acute resistance exercise bout.

nitrogen balance in 10 females during 7 days of endurance training (Roy *et al.*, 2002). Taken together, it seems that timing of nutrient ingestion should be a consideration in designing any post-exercise nutrition regimen. In summary, these studies indicate that, for any given amount of protein ingested, the timing of ingestion, as well as the composition of the protein or amino acids and other nutrients ingested concurrently, will determine the response of the athlete.

Summary and recommendations

There remains much to be elucidated about protein nutrition in athletes. Recent research suggests that quantifying protein to be ingested may be overly simplistic. Certainly, an adequate energy intake appears to be critical for an anabolic response on a chronic basis. If muscle hypertrophy is the primary goal for an athlete, then a hyperenergetic diet may be the most important recommendation. However, a parallel increase in fat mass may be an undesired effect of this approach and should be considered in light of the training and competitive goals of the individual athlete. Furthermore, a specific protein recommendation based solely on the quantity of protein ingested per day is dependent on the assumption that nitrogen retention, and thus muscle hypertrophy, is matched directly with protein intake. Clearly, regulation of the post-exercise response of net muscle protein balance to nutrient ingestion is much more complex than simple quantification of protein ingestion and many other factors (e.g. type and amount of amino acids, protein digestive properties, timing of nutrient intake in relation to exercise and other nutrients, concurrent ingestion of other nutrients and total energy intake) influence muscle and whole-body anabolism. Thus, for any given amount of protein, the response of protein anabolism will vary depending on exactly what is eaten and when.

Perhaps this issue can be examined another way. Since there is evidence that protein intakes above the RDA may be beneficial to athletes, a risk-benefit analysis may be useful. An important consideration is the potential harm that may arise from elevated protein intakes. There is little research into the maximum tolerable protein intake in healthy individuals. It has been suggested that excessive protein intakes may increase calcium loss, thus affecting bone health. However, since a major portion of bone is protein, excessive protein does not appear to influence bone health. High protein intakes have been suggested to pose a risk for the kidneys but, in healthy individuals with no underlying kidney disease (presumably most elite athletes), there is no evidence for harm to kidneys with higher intakes. Certainly, it would be detrimental for an athlete to consume excess protein at the expense of other nutrients required to support the necessary level of training and competition. There is a suggestion that intakes greater than 40% of total energy intake might be the upper limit. Protein intakes greater than 40% may limit intake of fat and/or carbohydrates, thus compromising the benefits of these nutrients. However, given the high energy intakes of most elite athletes, protein intakes higher than 40% are unlikely in most. Even a small female restricting energy intake and consuming only 1500 kcal would need to consume 150 g of protein to reach 40%.

Although there is evidence to the contrary, nitrogen balance methods identical to those used in sedentary individuals indicate that active individuals require more protein. Therefore, it can be argued that athletes may need more protein than sedentary individuals. Recommendations of 1.2–1.7 g protein \cdot kg⁻¹ BW \cdot day⁻¹ have commonly been made. There is no reason to suspect that intakes in this range would be harmful; however, the vast majority of athletes consume this amount of protein in their habitual diet, so recommending increased protein is unnecessary. It is important to remember that athletes and coaches are not that interested in the scientific arguments on both sides of this issue. Success in competition is what they consider important. It is common practice for athletes involved in strength and power sports to consume amounts of protein well in excess of the amount required to maintain nitrogen balance (Alway et al., 1992). The results of studies of the response of muscle protein metabolism to the intake of protein or amino acids suggest a beneficial effect of high protein intake in conjunction with resistance exercise. On the other hand, there is scant evidence from properly controlled studies of sufficient duration in power athletes to assess quantitatively the value of this practice in terms of gains

of muscle mass and strength. Thus, for athletes desiring muscle hypertrophy, there is little reason to limit protein intake and relatively high intakes might be the best recommendation. Of course, muscle anabolism may be enhanced by varying the type and timing of protein and amino acid ingestion. Even if 2.5-3.0 g protein \cdot kg⁻¹ BW \cdot day⁻¹ is consumed and this amount of protein is more than the synthetic machinery can process, the excess will simply be oxidized. As long as the intake of other nutrients important to the success of an athlete is not compromised, there appears to be little harm in ingesting these high amounts. Furthermore, this amount of protein may be considered to be reasonable given the high energy intakes in these athletes. For example, intakes of ~ 6400 kcal have been reported in strength athletes (Alway et al., 1992). Protein of only 14% of total kcal would be ~ 2.5 g protein \cdot kg⁻¹ BW \cdot day⁻¹ for a large athlete (~90 kg) and 3.2 g protein \cdot kg⁻¹ BW \cdot day⁻¹ for a smaller athlete $(\sim 70 \text{ kg}).$

The protein intake strategy should be tailored to the functional needs of the particular sport and, perhaps even more specifically, to the particular positional requirements within a sport and the individual needs of the athlete. It is unlikely that optimal protein intake for a marathon runner would be the same as for a power lifter. Muscle hypertrophy is rarely a positive factor for success for endurance athletes. Thus, if increased protein intake results in increased muscle mass or if the dietary protein replaces carbohydrates critical to replenishing glycogen stores, then the upper limit may necessarily be lower for endurance athletes. Thus, there appears to be no reason for recommending intakes above 2 g protein \cdot kg⁻¹ BW \cdot day⁻¹ and there is no evidence that intakes this high confer any advantage to an endurance athlete. In fact, Tarnopolsky et al. (1988) have clearly demonstrated that intakes above 1.7 g protein \cdot kg⁻¹ BW \cdot day⁻¹ simply result in oxidation of the ingested excess. Nevertheless, even 2 g protein \cdot kg⁻¹ BW \cdot day⁻¹ would only be about 18% of an endurance athlete's diet (based on a 70-kg athlete consuming 3000 kcal \cdot day⁻¹); hardly an excessive amount of protein to be eaten, and most endurance athletes regularly consume this amount of protein in any case. Thus, there is little reason to recommend intakes over and above that which is habitual for most athletes.

Many team sport athletes, such as those who play soccer, basketball, hockey and rugby, may desire both increased mass and endurance. Thus excessive protein intakes, such as those that may be ingested by strength athletes, are unlikely to be advantageous and muscle mass gains may best be sought by attempting to take advantage of timing of ingestion and composition of the proteins or amino acids ingested.

Finally, this review is not meant to advocate very high protein intakes for athletes, but merely to point out that it is possible, if not likely, that many athletes, especially those involved in strength and power sports, may benefit from higher intakes. However, as was concluded in the first consensus paper by Lemon (1991), most athletes habitually ingest sufficient protein, so recommending greater protein intakes does not appear warranted. Perhaps other factors, such as the timing of protein or amino acid intake in relation to exercise and the intake of other nutrients, are more important considerations for those athletes wanting to increase their body mass. Clearly, there is much to be learned about the optimal protein intake for elite athletes. There is a paucity of research examining the impact of various amounts of dietary protein on performance and body composition endpoints in top athletes. Clearly, longterm studies on the impact of different amounts of protein on performance variables, body composition as well as the metabolic and molecular mechanisms responsible for these changes in elite athletes would be valuable; however, proper control of such studies is virtually impossible given the small differences that must be detected. Although extremely difficult to control properly and to carry out, these long-term endpoint studies are necessary to determine definitively the protein needs of athletes and to overcome the controversy surrounding this issue.

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