

# PROTEIN AND AMINO ACID METABOLISM DURING AND AFTER EXERCISE AND THE EFFECTS OF NUTRITION

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Michael J. Rennie

*Department of Anatomy & Physiology, University of Dundee, Dundee DD1 4HN,  
Scotland; e-mail: m.j.rennie@dunde.ac.uk*

Kevin D. Tipton

*Division of Metabolism, Department of Surgery, University of Texas Medical Branch,  
Shriners Burns Hospital, Galveston Texas 77550; e-mail: ktipton@utmb.edu*

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This review is dedicated to Gail Butterfield, who died prematurely on Christmas day, 1999, and who with Doris Calloway wrote a classic paper on the subject of exercise and nitrogen balance.

■ **Abstract** Sustained dynamic exercise stimulates amino acid oxidation, chiefly of the branched-chain amino acids, and ammonia production in proportion to exercise intensity; if the exercise is intense enough, there is a net loss of muscle protein (as a result of decreased protein synthesis, increased breakdown, or both); some of the amino acids are oxidized as fuel, whereas the rest provide substrates for gluconeogenesis and possibly for acid-based regulation. Protein balance is restored after exercise, but no hypertrophy occurs with habitual dynamic exercise. Resistance exercise causes little change in amino acid oxidation but probably depresses protein synthesis and elevates breakdown acutely. After exercise, protein synthesis rebounds for  $\leq 48$  h, but breakdown remains elevated, and net positive balance is achieved only if amino acid availability is increased. There is no evidence that habitual exercise increases protein requirements; indeed protein metabolism may become more efficient as a result of training.

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## INTRODUCTION

There are several major reasons to study interactions between muscle protein metabolism during and after exercise and nutrition. Muscle contains a large proportion of the total protein in the adult body (40%) and accounts for between one third and one half of all protein turnover in the body. Its total mass and cellular and subcellular composition are markedly affected by the extent and type of its habitual contractile activity; furthermore, muscle is important not only as a machine for the transduction of chemical energy into mechanical work, but it is also engaged in the diurnal regulation of the ebb and flow of amino acids between the center and the periphery with feeding and fasting, and muscle can be considered to be a store of energy and nitrogen during starvation and disease and after injury. The mechanical role of muscle impinges metabolically on all of these aspects, as well as upon the regulation of glucose tolerance, the partition of the fat and lean body masses, and the maintenance of the lean body mass throughout middle and old age. Finally, of course, there is a huge interest among amateur and professional sports people and the military in the optimal type of nutrition for better performance, whether it is to increase endurance or promote muscle size and strength. As a result, there is still a large amount of wishful thinking concerning the effects of food and food supplements on exercise performance and muscle

building and the effects of exercise on protein requirements for physically active people.

This review deals with basic metabolic and nutritional physiology as it affects muscle and attempts to provide a framework for answering some of the important practical questions concerning exercise and nutrition. It extends rather than revisits most of the material covered in a recent comprehensive review written from a more purely physiological perspective (87).

## CHARACTERISTIC FEATURES OF MUSCLE COMPOSITION AND FUNCTION RELATED TO PROTEIN AND AMINO ACIDS

Actin and myosin are among the most abundant proteins in the mammalian body, and they are chiefly found in muscle, accounting for ~65% of the total muscle protein. Thus their primary amino acid composition has a substantial effect on the composition of the amino acids released from protein in muscle during protein breakdown and withdrawn from the free pool during protein synthesis. Particularly striking is the large proportion of the branched-chain amino acids (BCAAs), leucine, valine, and isoleucine, which together account for 20% of the total amino acids released from protein. Muscle contains all of the other essential amino acids, which, of course, is why it is such a valuable food.

A useful concept in muscle protein metabolism is the relationship between the amount of an amino acid in protein and that in the free muscle pool, which indicates which amino acids might be limiting for protein synthesis, including the BCAAs and possibly methionine and tyrosine.

Muscle as a tissue shows a number of important characteristics that influence its metabolic behavior related to protein. First, once fully differentiated, the number of muscle cells stays constant (within a few percent), so growth and wasting, with a few exceptions, can occur only through hypertrophy or atrophy of existing muscle cells. Second, the fiber type composition of muscle [i.e. the number of fibers of types I, IIa, and IIb (see 101 for review)] appears to be genetically determined, although the phylogenetic expression of muscle proteins is under the control of the type and amount of contractile activity. Studies of the development and adaptation of muscle to chronically altered activity, each of which requires modification of the expression of a different array of cellular components, suggest that a common suite of nuclear controllers is involved, with programmed responses turned on or off by nuclear transcription factors (see 97 for review).

Muscle possesses all of the common apparatus for protein turnover, although not necessarily in the forms expressed elsewhere. Skeletal muscle is a syncytium, with nuclei shared between cells; its ribosomes are not found arrayed on endoplasmic reticulum. It does not seem to possess morphologically well-defined lysosomes, such as are seen in other tissues, but nevertheless there is a full complement of

lysosomal (105) and extra-lysosomal proteolytic activities (e.g. the ATP-dependent ubiquitin pathway) (3), which are important in degrading muscle protein.

In animal muscles, the slow-twitch, more oxidative muscle fibers have a greater rate of protein turnover than the faster-twitch, less oxidative, more glycolytic fibers, but it has not been determined definitively whether this is true for human muscle. The current, very limited evidence suggests that it is not (75, 99).

## AMINO ACID TRANSPORT, SYNTHESIS, AND CATABOLISM IN SKELETAL MUSCLE

### Transport

The size and composition of the free amino acid pool in muscle are net results of inward and outward transport of amino acids, as well as their disappearance into and appearance from protein (i.e. protein turnover) and the *de novo* synthesis and catabolism of the amino acids. Muscle apparently has a full complement of amino acid transporters, similar to those existing elsewhere in the body (106). The kinetic characteristics and ionic and hormonal sensitivities of these transporters have been well described and appear to offer little help in understanding regulation of amino acid metabolism at rest or during and after exercise (87). Generally speaking, because of the low affinity of the transporters concerned, amino acid transport operates at rates that are directly proportional to the prevailing amino acid concentrations inside and outside the sarcoplasmic membrane. The rates are sufficiently high that the activities of other metabolic processes are not limited, such as protein synthesis or BCAA oxidation. There are some exceptions, such as the transport of glutamate, which is saturated at low, near-plasma concentrations and which has such a low maximum capacity that plasma and intramuscular-glutamine pools are effectively separated. This has important implications for regulation of glutamine synthesis and control of concentration of Krebs cycle intermediates, both of which require intracellular glutamate.

### Intermediary Metabolism

Intermediary metabolism of amino acids in muscle is somewhat limited. Glutamate, alanine, and aspartate may be synthesized in the sarcoplasm from by-products of carbohydrate catabolism with appropriate transamination; glutamine is synthesized from glutamate and ammonia by glutamine synthetase. Most amino acid catabolism (which is limited in scope compared with that occurring in liver or kidney) is mitochondrial; the transamination and decarboxylation of the BCAAs is overwhelmingly mitochondrial (102), as are glutaminase and glutamate dehydrogenase. The branched chain ketoacid dehydrogenase enzymes, which rapidly decarboxylates the ketoacids to CO<sub>2</sub> and the appropriate hydroxy acid, resemble pyruvate dehydrogenase in structure and regulation. They are stimulated by Ca<sup>2+</sup> and their substrate, and they are inhibited by insulin, as a result of covalent modification; phosphorylation diminishes the activity (42, 54).

Methionine may be transaminated and is also subject to transsulfuration in muscle (98), but the pathway is quantitatively unimportant. No other amino acids are subject to intermediary metabolism in muscle, although all may take part in protein turnover.

## Diurnal and Exercise Modulation of Amino Acid Metabolism

The size and composition of the muscle free amino acid pool depend upon the body's nutritional state, the plasma amino acid availability, and the hormonal milieu. At rest, immediately after a mixed meal, the delivery of amino acids to muscle exceeds its capacity to deposit them as protein. The intramuscular amino acid pool is expanded (10) but not by as much as might be expected, because of protein synthesis, the inhibition of breakdown, and the stimulation of the BCAA-catabolizing enzymes, which are supply driven, given the high values of their  $K_m$ s. The BCAAs are rapidly transaminated, and, in the presence of ample pyruvate (from blood glucose), synthesis of alanine is stimulated; glutamine production also increases but not to the same extent. The net balance of other nonmetabolized amino acids simply reflects the protein balance.

As the post-absorptive state progresses, protein synthesis turns down, and breakdown is accelerated; in these circumstances, in which glucose uptake into muscle is minimized and glycogenolysis proceeds slowly, alanine production gives way to glutamine production, with most of the nitrogen coming from BCAAs (as well as alanine and glutamate) released from muscle protein breakdown. All of the de novo-synthesized alanine carbon comes from pyruvate, and the carbon for glutamine comes from  $\alpha$ -ketoglutarate via glutamate. In turn,  $\alpha$ -ketoglutarate comes mainly from the carbon skeletons of valine and part of isoleucine, which can enter the Krebs cycle at succinate. All three amino acids are decarboxylated, but only leucine is completely oxidized in the Krebs cycle, because it is the only one that gives rise to acetate. This may have important implications when anaplerotic maintenance of tricarboxylic acid intermediates is limited during long-term exercise or during excess availability of BCAAs. Some BCAA carbon (from valine and isoleucine) may escape muscle as hydroxy acids, thereafter contributing to gluconeogenesis (19).

The fates of alanine and glutamine are mainly gluconeogenesis and ureagenesis (29, 77). At rest, in the post-absorptive state, muscle amino acids may account for 30% of total gluconeogenesis, and the amount rises during exercise, although gut-derived amino acids may contribute substantially as exercise continues (117, 121).

During moderate to severe dynamic exercise, alanine and glutamine production rises, as does the production of ammonia and BCAA oxidation. These phenomena can be explained as the combination of increased delivery to working muscle of plasma BCAAs, increased protein breakdown, increased availability of pyruvate, and possibly increased activity of mitochondrial glutamate dehydrogenase. Certainly, intramuscular free-glutamate concentrations fall during dynamic exercise, possibly as a result of increased transamination to alanine and glutamine synthesis, but also because of increased glutamate dehydrogenase activity (94). This would

tend to maintain the steady-state concentration of the Krebs cycle intermediates, which would help maximize oxidative use of fuels.

The activity of the malate-aspartate shuttle, which transports reducing equivalents into the mitochondria, is stimulated during exercise, but this has no net effect on amino acid pools. The purine nucleotide cycle, which produces ammonia and is potentially a net user of aspartate and thus possibly of N coming from BCAAs, appears to be markedly active only after exercise (53).

Muscle tissue possesses the inducible form of nitric oxide synthase, which is responsible for observed increases in NO production in muscle during exercise. There is now good evidence that modulation of metabolism by the NO system occurs, with most information being available concerning regulation of glucose transport (5). However there seem to have been few investigations of possible effects on amino acid transport or metabolism or protein turnover. Muscle soreness associated with increased muscle damage (and stimulation of net loss of muscle protein) due to eccentric contraction seems to be NO mediated (84). Also the stimulatory effect of insulinlike growth factor-1 on protein synthesis is abolished by an NO synthesis inhibitor, suggesting a role for the system in regulation of the anabolic effect of insulinlike growth factor-1 (41).

## Amino Acids and Fuel Metabolism in Muscle

The effects of bouts of resistance exercise upon muscle amino acid intermediary metabolism have not been studied in detail, partly because the periods of work are too short to result in more than bursts of increased rates of ATP utilization and glycogenolysis, with little oxidative metabolism being involved; the meager evidence available suggests that there is little excess amino acid oxidation (104).

In dynamic exercise such as sprinting or cycling at power outputs of  $\sim 70\%$   $\dot{V}O_{2\max}$  and above, there is a substantial activation of glycogenolysis, glucose transport, and the tricarboxylic acid cycle. The major fuels are pyruvate and fatty acids, and the quantitative contribution of amino acids is small and proportional to work load. Below we summarize current knowledge only to the extent that it provides a framework for discussing new findings. Interested readers wanting to read more about the subject are referred to previous reviews (50, 53, 87, 115). Exercise enhances glutamate and BCAA uptake from the blood (1, 38), and alanine and glutamine production increases almost linearly with aerobic power, whereas ammonia production seems to show a more exponential relationship, like lactate production (48, 49). Muscle amino acid concentrations show little change with short-term exercise or exercise at power outputs below  $70\%$   $\dot{V}O_{2\max}$ , but thereafter the intramuscular concentration of glutamate falls sharply (59). In prolonged exercise or exercise at high rates of power output, the normal post-absorptive net negative amino acid balance is increased with increased production of amino acids that are not metabolized in muscle (e.g. the aromatic amino acids, lysine, and threonine) (16). Whether this is caused by increased protein breakdown, decreased protein synthesis, or both is not completely resolved.

Since the major review of this subject in 1996 by one of us (87), the most interesting developments have concerned the further elucidation of the metabolism of BCAAs in muscle (69, 112, 113), the possibility of alterations of the pool sizes of the components of the Krebs cycle [i.e. anaplerotic replenishment (45, 46, 51)], and the possible interrelationship between the two.

Because leucine transamination removes  $\alpha$ -ketoglutarate and the leucine carbon is completely oxidized to  $\text{CO}_2$  without any replenishment of the Krebs cycle intermediates (unlike valine or half of isoleucine), and if pyruvate is limiting during exercise, possibly as a result of decreased glycogen stores, Wagenmakers has postulated that the Krebs cycle flux would be limited (114, 115). It seems to be agreed that, at the beginning of exercise, the source of carbon for expansion of the cycle intermediates is glutamate converted via the alanine aminotransferase reaction, and, indeed, when pyruvate is diverted to acetyl coenzyme A formation, the concentration of intermediates falls. Also it is agreed that availability of BCAAs and exercise activates the branched-chain ketoacid dehydrogenase complex (112) and, at rest at least, the complex is more activated under conditions of decreased muscle glycogen availability. However, European and Canadian groups disagree about the effects of exercise. The Europeans contend that the activation of the dehydrogenase is maintained at a higher level in glycogen-depleted muscle, which might result in a higher rate of leucine oxidation and a greater possible depletion of Krebs cycle intermediates (115a). However, the Canadians are unable to detect any greater stimulation on the branched-chain ketoacid dehydrogenase (55) and no differential effect of availability of either glycogen or BCAAs on the concentration of Krebs cycle intermediates (44).

Furthermore, it now seems certain from the weight of accumulated evidence that ingestion of BCAAs does not lead to any marked effect on performance—either enhancement or diminution (68)—casting doubt on the hypothesis of Wagenmakers et al (116) of increased use of BCAAs under conditions of low glycogen availability. In fact there is no good published evidence of a beneficial effect of BCAAs on exercise performance as a result of their use as a fuel, and we must conclude that there is none.

There is some contention concerning the effects of training on BCAA metabolism. It has been reported that, in endurance-trained rats, not only is the total activity of the branched-chain ketoacid dehydrogenase enhanced but the enzyme responsible for inactivation of the complex (a protein kinase) is less abundant, explaining why the apparent activation response during exercise is greater (42). Because training increases muscle mitochondrial density, increasing the total capacity of the branched-chain ketoacid dehydrogenase enzyme, the extent of leucine oxidation during exercise at a given work rate ( $50\% \dot{V}\text{O}_{2\text{max}}$ ) is greater in trained than untrained human beings during exercise (62). However, when expressed per lean body mass, the training effect disappears, suggesting a lesser activation of the greater capacity for leucine oxidation at the same relative workload.

When glycogenolysis and lipolysis are prevented by  $\beta$ -adrenergic blockade (61), which reduced the availability of glucose and free fatty acids in blood during

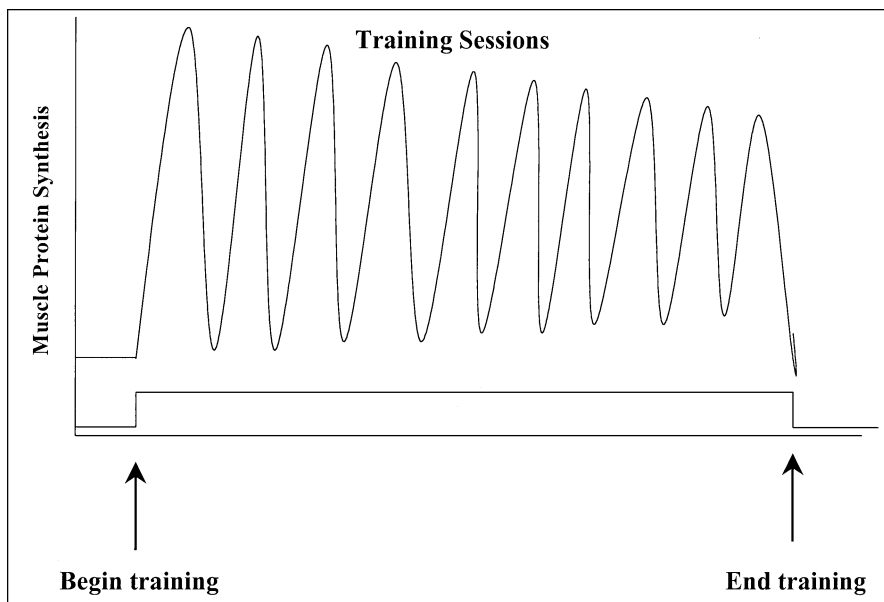
exercise, leucine oxidation was increased markedly, thus suggesting that, under normal circumstances, leucine is not a favored fuel, and its use is suppressed by other fuels, if available in sufficient amounts.

## Extramuscular Metabolism

During exercise, blood flow is diverted to working muscle from other organs, but, nevertheless, the diversion is not sufficient to shut down important metabolic processes in gut, liver, and kidney. Indeed there is now good evidence that the gut switches from absorbing nutrients to supplying amino acids to the periphery (121). In dogs in the fasted state, net proteolysis in the liver and the gut both contribute to whole-body availability of amino acids. However, during moderate treadmill exercise, the amino acid production from liver diminishes, and that from the gut increases. The fate of the amino acids released is likely to differ according to their nature. Muscle will oxidize glutamate and the BCAAs, and the liver will use the rest to produce ketone bodies and glucose according to type. Presumably, given the close linkage between ureagenesis, gluconeogenesis, and ketogenesis, all three processes are coordinately regulated during exercise. Some new information about this regulation comes from studies in normal healthy men, in which somatostatin was used to inhibit pancreatic hormone secretion, with replacement on different occasions of insulin or glucagon or both together (64). The results indicate that the activation of liver ureagenesis and gluconeogenesis by glucagon must play a very important part in their exercise-induced stimulation. In the absence of glucagon, no increase at all occurred in hepatic glucose production or gluconeogenesis. The absence of insulin in the presence of glucagon caused a marked stimulation of hepatic glucose production and gluconeogenesis. The results strongly suggest that elevated delivery of amino acids alone (most likely from increased gut and muscle protein breakdown) is not enough to stimulate gluconeogenesis. Furthermore, activation of the liver's catabolic potential for amino acids may lower plasma amino acid concentrations sufficiently to stimulate net loss of amino acids from muscle, through either a diminution of protein synthesis or a rise in breakdown.

## RESPONSE OF MUSCLE PROTEIN SYNTHESIS TO AN ACUTE EXERCISE BOUT

It is now clear that an acute bout of exercise has a dramatic effect on the rate of muscle protein synthesis. The size, direction, and timing of the effect seem to depend on several factors, including the intensity, duration, and mode of exercise. The proximate mechanical stimuli that lead to changes in muscle protein synthesis are unknown, but they are likely to include tension (i.e. force generated), stress (i.e. force per unit area of the muscle), and energy used (indicated by e.g.  $\dot{V}O_2$ ) or mechanical work done (i.e. the force-time integral) and possibly combinations of these (Figure 1). The cellular signals that transduce these factors remain to



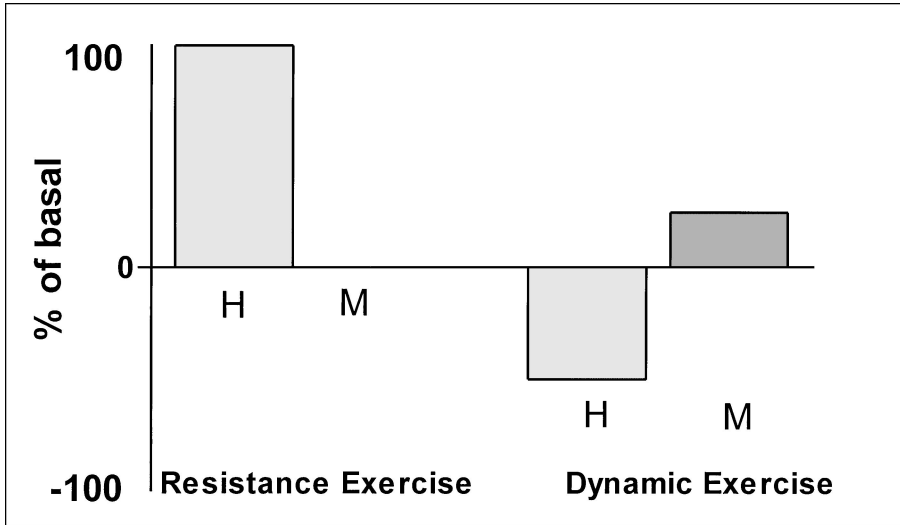
**Figure 1** Hypothetical response of muscle protein synthesis to repeated workouts during a resistance exercise training program.

be elucidated, although cell membrane stretch may be an important sensed variable with cellular correlates such as increased focal adhesion kinase and paxillin activity (25, 26). A muscle-specific nonglycosylated variant of insulinlike growth factor-1 also appears to be important in the remodeling responses to stretch in skeletal muscle (47). There has been a considerable amount of research over the last few years to assess the response of muscle protein synthesis during and after exercise.

### Effects of Exercise During Ongoing Contraction

It is difficult to make measurements of protein turnover over short periods of time with isotope tracers, because of problems in achieving and maintaining isotopic steady states, getting sufficient incorporation for accurate measurement of enrichment of protein, and knowing the labeling of the appropriate precursor pools, etc. Thus, much of our information about changes in protein synthesis and breakdown during exercise is only semiquantitative or is an interpretation of indirect indices.

It seems reasonable to predict, *a priori*, that if better methods were applied, the results would show that, if exercise were intense enough and/or long enough, muscle would show acutely a net negative protein balance. Extrapolating from animals, the mechanisms would likely be a fall in protein synthesis and a rise in protein breakdown. In the only study addressing this, Carraro and colleagues did indeed see a fall in human muscle protein synthesis during 4 h of walking (Figure 2) (24).



**Figure 2** Relative change of muscle protein synthesis from basal after high (H) and moderate (M) intensity resistance and dynamic exercise.

In human muscle it is difficult to see any marked increase in net loss of amino acids during dynamic exercise unless it is at high intensities, that is, probably  $>70\% \dot{V}O_{2\max}$  (38, 109). Also, apart from the alterations in the pool sizes of those amino acids metabolized in muscle, there are no marked alterations in muscle free-amino-acid pools during moderate exercise, which suggests that little change occurs in muscle protein turnover during this type of activity. However, when one-legged exercise (which places a much bigger metabolic strain on muscle) is performed, the net negative amino acid balance becomes more marked (16, 110). Work in running rats suggests that a high intensity of exercise is needed to stimulate net loss of amino acids and that myofibrillar protein breakdown is not necessarily stimulated (34).

## Changes After Exercise

**Resistance Exercise** Generally, the rate of mixed muscle protein synthesis is increased after resistance exercise in both human beings and rats (13, 15, 36, 37, 39, 40, 122). There are some reports of a lack of any response of muscle protein synthesis to resistance exercise in human subjects (93, 108). However, the non-responding subjects in both studies were already resistance exercise trained, and what was observed might be expected of a successful biological adaptation to a stress event. We postulate that whenever the stimulus is sufficient, that is, beyond the current capacity of the subjects, contractile activity always stimulates muscle protein synthesis. This occurred in the first ever reported study in human beings by

Chesley and colleagues (27), who found a 50% increase in muscle protein synthesis immediately after resistance exercise. The previously trained subjects in that study performed 12 sets of isolated biceps brachialis exercise at 80% of one repetition maximum (1RM), that is, more than their previous capacity. On the other hand, subjects who performed a whole-body routine with only six sets of exercises designed to work the deltoid at only 65% of 1RM showed no significant effect in that muscle group (108). Nevertheless, intensity alone may not be enough. The intensity of exercise performed by subjects in the study of the vastus lateralis by Roy and colleagues (93) was high (85% 1RM), but these subjects performed only four sets of an exercise involving the sampled muscle.

It is worth noting that in human muscle, when the response occurs, the increase is usually large, that is,  $\geq 50\%$ . The effect seems to persist for  $\leq 48$  h before abating (67, 80). Because the changes in muscle protein mass are always much less than 50%, it is obvious that muscle protein breakdown must be stimulated (see below). This would be a reasonable adaptive biological response involving some cellular remodeling.

**Dynamic Exercise** Dynamic exercise seems to have a different effect from resistance exercise on muscle protein synthesis. In most studies of rats, muscle protein synthesis was reduced below the resting rate after intense dynamic exercise [running at  $\sim 75\%$ – $85\%$  of  $\dot{V}O_{2\max}$  for  $\geq 2$  h or swimming from 1 h to exhaustion (2, 32, 33, 43)]. Furthermore, the longer or more intense the exercise bout, the more protein synthesis fell. Thus, after running 28 m/min for 1 h, a subject's gastrocnemius muscle protein synthesis fell by 30%, but after running to exhaustion, the rate was reduced by 71% (32, 33). Rats running at a mild intensity showed no significant decrease in muscle protein synthesis (6). It thus seems likely that dynamic exercise reduces mixed muscle protein synthesis in an intensity-dependent fashion.

Muscle protein synthesis in human beings after dynamic exercise appears to have been investigated in only two studies. Tipton and colleagues (108) could find no significant increase in deltoid muscle protein synthesis after an intense 1.5-h swimming workout, but on the other hand, Carraro and colleagues (24) found a 25% increase in protein synthesis in the vastus lateralis after 4 h of walking at 40%  $\dot{V}O_{2\max}$ . The discrepancy between the two studies is puzzling. Although the walking exercise bout was prolonged, the intensity was relatively low, and the vastus lateralis is not likely to have been stressed to the same extent as the deltoids of the swimmers in the previous study. However, the swimmers were highly trained, so they may have become adapted. There are no reports on studies of the influence of training on the response to a bout of dynamic exercise in people.

## Time Course of Effects

Resistance exercise seems to have an effect on human muscle protein synthesis for  $\leq 48$  h (67, 80), but there is no information on the time course of the response to

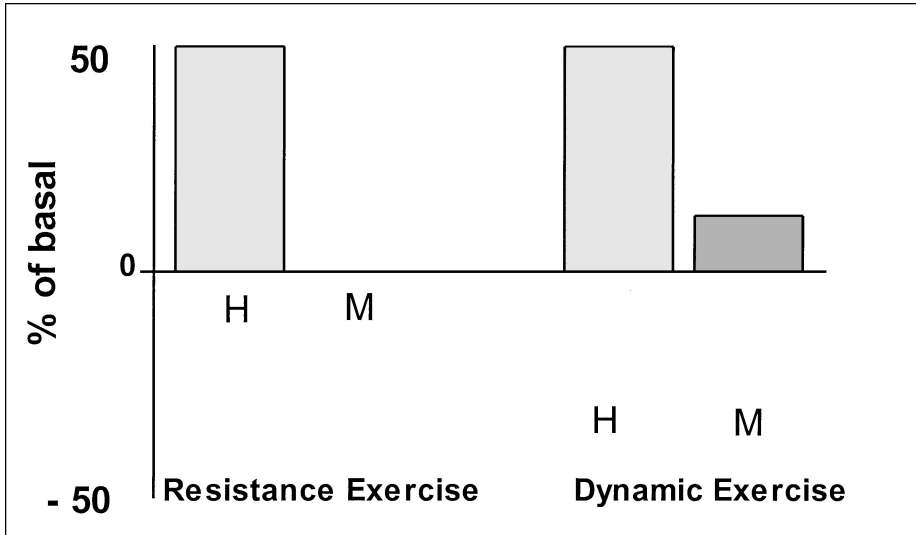
dynamic exercise. In rats, in which high-intensity, dynamic exercise had reduced muscle protein synthesis below control rates immediately after exercise, the rates returned to control values in a matter of hours, depending on the intensity and duration of the exercise bout (2, 32, 33, 43). The more severe the exercise, the slower the recovery of muscle protein synthesis.

## RESPONSE OF MUSCLE PROTEIN BREAKDOWN TO AN ACUTE EXERCISE BOUT

The response of muscle protein breakdown to exercise has not been extensively studied. Some studies reported postexercise increases in the rates of muscle protein breakdown, both in rats and in human beings (32, 33, 89), based on an increase in the rate of excretion of 3-methylhistidine, an amino acid post-translationally produced in actin and myosin and secreted largely unchanged after protein breakdown. Others have reported different responses, that is, no change in (24, 82, 83) or decreased excretion of (74, 85) 3-methylhistidine in response to exercise. This variability illustrates one of the practical problems associated with the use of 3-methylhistidine as a marker of skeletal muscle actin and myosin breakdown. The other is the contribution from smooth muscle to whole body excretion (90).

Only measurements of protein breakdown based on tracer dilution are likely to be robust. Two such methods have been applied to measure muscle protein breakdown *in vivo* in humans. One of these, a three-compartment model using values of labeling and concentrations of amino acids in arterial and venous blood, as well as the labeling of the tissue free tracee (11), has been used to quantify muscle protein breakdown after a bout of heavy leg resistance exercise (13, 15). Muscle protein breakdown was increased after resistance exercise, but to a smaller extent than muscle protein synthesis. Another approach used a precursor product method that determined the decay in enrichment of labeled phenylalanine in the venous blood and the muscle intracellular free-amino-acid pool after cessation of the infusion of tracer amino acids as a measure of the dilution of tracer by amino acid from protein breakdown (127). When applied after intense knee extension exercise, the rate of muscle protein breakdown was found to be increased by ~50% in untrained human subjects (Figure 3) (80, 81).

There are currently no reports of studies of direct measurement of muscle protein breakdown after dynamic exercise in human subjects. However, rat muscle has been studied both *in vivo* (treadmill or swimming exercise) and *in situ*, and the results suggest that muscle protein breakdown is increased. However, degradation of myofibrillar proteins does not appear to contribute to the increase in total muscle protein breakdown after dynamic exercise. Whereas the release of 3-methylhistidine, a marker of myofibrillar protein breakdown, in perfused rat hindquarters was not different from controls, the release of tyrosine, a marker of total muscle protein breakdown, was increased (57, 58).



**Figure 3** Relative change of muscle protein breakdown from basal after high (H) and moderate (M) intensity resistance and dynamic exercise.

The stimulation of muscle protein breakdown, as for synthesis, is transient. In rat muscle assayed *in vitro*, the stimulation of muscle protein breakdown after exercise is observable for only 2–3 h (30). In human beings, muscle protein breakdown was elevated after resistance exercise at 3 and 24 h postexercise, returning to basal rates by 48 h afterwards (80).

## CONNECTIVE TISSUE METABOLISM AND EXERCISE

Measurements of markers of collagen turnover (such as procollagen peptides as anabolic indices, and C- and N-terminal telopeptides as catabolic indices) in arterial and venous blood sampled across the human leg suggests that exercise is acutely catabolic for collagen (18). This is consonant with a large body of work on muscle collagen metabolism in rats, which demonstrates that running exercise, especially downhill running, which causes eccentric contractions (i.e. stretching of muscle during contraction), inhibits collagen synthesis at the time and markedly enhances its breakdown, with opposite compensatory or adaptive changes occurring in recovery (52). More detailed confirmation of this pattern in nonmuscle connective tissue comes from elegant studies with microdialysis of the peritendinous space of the Achilles tendon in runners before, immediately after, and 72 h after 36 km of running (63). The pattern of changes of a depression of synthesis and stimulation of catabolism during exercise and stimulation of both afterward, with the anabolic process predominating, seems to be a common response shared by muscle and connective tissue.

## RESPONSE OF MUSCLE PROTEIN METABOLISM TO EXERCISE TRAINING

Exercise training of particular kinds clearly has a dramatic impact on muscle morphology and function compared with the effects of weight lifting and distance running on fiber type diameters and proportions or mitochondrial density. It is no surprise that different modes of training, aimed at increasing the performance of particular kinds of muscular activity (or, in bodybuilding, simply muscle mass) have differential effects on muscle protein metabolism. Resistance exercise training is well known to result in hypertrophy of the trained muscles (56, 66). Common sense suggests that bodybuilders and athletes who wish to develop muscle mass should lift weights rather than run long distances. From this, we can predict that resistance exercise training should produce much more of a stimulatory effect on net muscle protein balance than dynamic exercise training. However, the capacity of dynamic exercise training to increase muscle strength and muscle fiber cross-sectional area does exist (28, 60, 76, 95), depending on the relative intensity and duration and the mix of the two modes used. Thus we might expect some stimulation of net muscle protein accretion with dynamic exercise training of a sufficient intensity.

The effects of exercise training on human muscle protein synthesis and breakdown have not been extensively studied, with relatively few reports on the phenomenology and fewer still on mechanisms. Most of the data extant concerns the response to resistance exercise training.

It must be true that muscle hypertrophy resulting from exercise training is caused by an increase in muscle protein synthesis in the resting recovering muscle, but an important question is the extent to which the basal rate of resting protein turnover is elevated. Assuming that muscle protein synthesis responds to a repeated stimulus of a constant size (e.g. applied in a square-wave pattern) as do other physiological systems (e.g. maximal oxygen consumption capacity and so-called aerobic power)  $\dot{V}O_{2max}$ , then it is likely that a series of training stimuli will result in a series of responses each of which is progressively reduced (Figure 1). Thus, after training, an increased stimulus should, theoretically, be necessary to initiate a response similar to that observed before training.

The results from a cross-sectional study in resistance-trained and untrained human volunteers are consistent with this idea. When the response of muscle protein turnover was compared in trained vs untrained subjects (three males and females per group), both at rest and after intense resistance exercise, protein turnover was greater in the exercised than control leg, but the increase in both muscle protein synthesis and breakdown in the trained subjects was reduced (~50%) compared with that in the untrained subjects. There were no differences in the resting values.

The lack of a long-term stimulation of muscle protein turnover has also been observed in elderly subjects, who have a reasonable response to single bouts of exercise, but nevertheless show no change of the rate of incorporation of [ $^{13}C$ ]leucine in the resting values after a substantial period of training (118).

Further evidence of a down-regulatory adaptation comes from the work of Farrell and colleagues (35), who reported that postexercise muscle protein synthesis in rats that had been resistance trained for 8 weeks was actually reduced compared with that in untrained rats. In addition, muscles from rats trained for 4 months by running on a treadmill (73) or swimming for 4 weeks (30, 31) showed no increases in turnover.

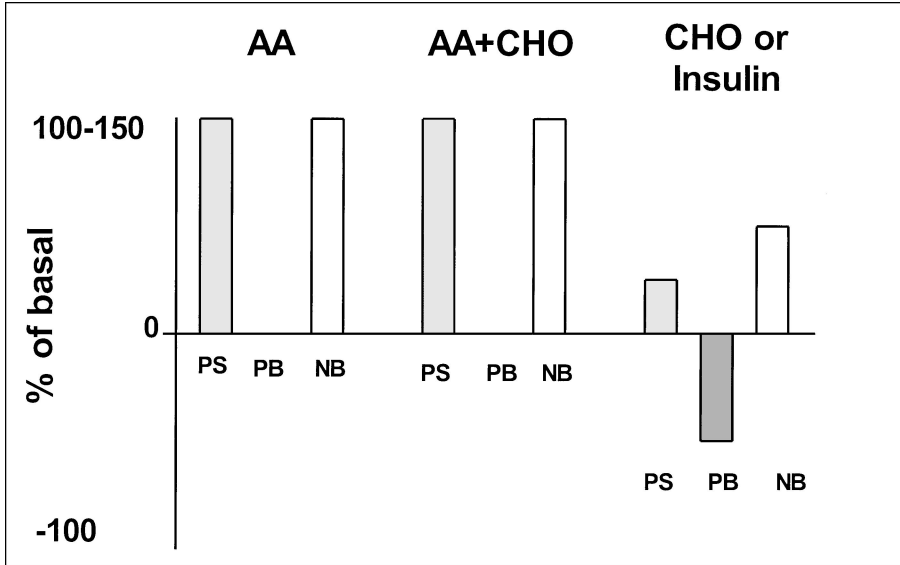
Most results point toward training-induced muscle growth as a result of an accumulated set of anabolic responses to each individual exercise training session, rather than as a result of a change in the relationship of muscle protein synthesis to breakdown at rest. However some reports do not fit this pattern. A series of reports (123–125) concluded that resistance exercise training, even for as little as 2 weeks, increased resting muscle protein synthesis in both young and old humans. It may be that, in the elderly, the basal rate of protein turnover is reduced by lack of activity and that this is normalized by training, but the effects in the young subjects are harder to understand. It is possible that the findings may have been confounded by the fact that whereas the initial, pretraining measures were made at rest, the final, post-training measures were made within 24 h after the last bout of resistance exercise. Because the response of muscle protein synthesis to such a bout of resistance exercise has been shown to last for 24–48 h (27, 80), it would probably be difficult to differentiate the response of muscle to the chronic effect of training from the response to the last acute exercise bout. In other words, any post-training increase in basal muscle protein synthesis is likely to simply be a hangover from the last workout bout, rather than the chronic effect of training.

## INFLUENCE OF NUTRITIONAL SUBSTRATES ON MUSCLE PROTEIN METABOLISM AFTER EXERCISE

When considering the consequences of exercise and exercise training on muscle, it is important to bear in mind the interactions of contractile activity and feeding. An acute bout of resistance exercise increases muscle protein synthesis more than breakdown, so that net muscle protein balance is increased. However, in the absence of food intake, the net response of muscle protein metabolism to an acute bout of exercise remains negative; that is, breakdown exceeds synthesis, and hypertrophy could occur only if feeding were to occur  $\leq 24$ –48 h after exercise.

Feeding a mixed meal causes an increase in the availability of substrates and hormones. At resting condition, the major modulators of protein turnover are amino acid availability (which has a large stimulatory effect on protein synthesis and a small effect on breakdown) and insulin (which has a small effect on synthesis and a large inhibitory effect on breakdown) (Figure 4) (7–9, 12).

Amino acid availability plays an important role in the control of muscle protein metabolism. Hyperaminoacidemia at rest has an anabolic effect on muscle, primarily by stimulating muscle protein synthesis (7–9). When Biolo and colleagues



**Figure 4** Relative change of muscle protein synthesis (PS), muscle protein breakdown (PB), and net muscle protein balance (NB) after exercise, when subjects are fed amino acids (AA), amino acids plus carbohydrates (AA+CHO), or carbohydrates alone (CHO or insulin).

examined the effect of a mixed amino acid infusion after exercise, this resulted in an enhanced rate of muscle protein synthesis compared with hyperaminoacidemia at rest (14). Thus amino acids and exercise seem additive in effect. Furthermore, during hyperaminoacidemia after exercise, muscle protein breakdown, which is normally elevated, did not rise, thus promoting net anabolism.

Intravenous infusion of amino acids is not a practical method for delivering amino acids to free-living human beings. However, first-pass splanchnic extraction of amino acids accounts for a large portion (20%–90%) of ingested amino acids, depending on the type (70, 71). Hence, it was not clear that oral delivery of amino acids would result in an increase that was sufficient to stimulate muscle anabolism to the same extent as an infusion. Also, because splanchnic protein breakdown is increased during exercise (121), it is possible that splanchnic extraction of amino acids is enhanced afterwards, as replacement. To resolve this, the response of human muscle protein turnover to ingestion of mixed amino acids, in an amount similar to that previously infused, was examined after resistance exercise (107). Postexercise net muscle protein balance was negative with placebo ingestion, but was positive during ingestion of amino acids, primarily through an increase in muscle protein synthesis. Thus, oral amino acids are clearly effective at promoting net muscle protein accretion. Any meal containing sufficient amino acids that is consumed within 24 h of resistance exercise would result in net muscle protein accumulation.

In the elderly, although the basal rate of turnover is depressed, the capacity to respond to exercise by increasing muscle protein synthesis is proportionately as great as in the young (120); however, a convincing study of the effect of varying the protein composition of meal suggests that the elderly cannot further increase protein synthesis in response to a high-protein meal (119).

The effect of consuming carbohydrates or fats on postexercise muscle protein metabolism has received only limited study. Carbohydrates alone resulted in no increase in muscle protein synthesis in rats after treadmill exercise (43). Also,  $\leq 2$ g/kg body weight of carbohydrates consumed immediately after exercise, causing a substantial increase in insulin secretion, did not significantly increase postexercise muscle protein synthesis in human beings (93). Muscle protein breakdown was not directly measured, but 3-methylhistidine excretion, an index [although not a quantitatively accurate one (90)] of myofibrillar protein breakdown, was less after carbohydrate than placebo ingestion. This finding is consistent with the idea that the postexercise increase in muscle protein breakdown may have been reduced by insulin (15), whose secretion was stimulated by the ingested carbohydrate.

The question of the role of insulin postexercise is difficult to resolve. Insulin availability falls with dynamic exercise probably owing to  $\beta$ -adrenergic inhibition of secretion, but the effect is much less, if measurable at all, with resistance exercise. During recovery, insulin secretion rebounds to above basal values. Although glucose ingestion is a reasonable stimulator of insulin secretion, the amino acids, in the dose sizes given after exercise in studies to date, are poor secretogogs. Thus, it is likely that, unless a large carbohydrate-containing meal is taken after exercise, insulin availability would be relatively low. There is good evidence of an inhibition of muscle protein breakdown postexercise, but, as for protein synthesis at rest, the demonstration of a stimulatory effect of insulin alone on postexercise protein synthesis is difficult. The problem arises because of what appears to be the acute sensitivity of protein synthesis to amino acid availability. Unless amino acids are given concomitantly with insulin or insulin is infused very near the site of its required action to reduce systemic effects, providing insulin reduces protein breakdown and inhibits protein synthesis via a fall in amino acid availability. Thus, no further increase in protein synthesis could be observed postexercise when insulin was given (15). One possible explanation is that insulin and contractile activity ultimately use the same signaling pathway to stimulate synthesis and that the stimulation was maximal.

Insulin may also have played a permissive role at low concentrations in the stimulation of muscle protein synthesis after exercise. The presence of physiological levels of insulin is necessary for an increase in protein synthesis to occur after resistance exercise in rats studied *in situ* (39,40). Apparently, the concentration of insulin required to allow the postexercise stimulation of muscle protein synthesis to occur is relatively low and may be inversely dependent on exercise intensity. Thus, when partially diabetic rats, able to produce only small residual amounts of insulin, were studied after exercise, they had normally increased rates of muscle protein synthesis some 16 h after exercise; it is interesting that, when

the intensity of exercise was increased, this increased protein synthesis was not observed (36).

Clearly, amino acids are necessary for net muscle anabolism after exercise by stimulating muscle protein synthesis. The presence of insulin allows muscle protein synthesis to proceed, whereas higher levels seem to diminish the postexercise response of muscle protein breakdown (15). Thus, a combination of amino acids and carbohydrates, to stimulate insulin release, may be the optimal postexercise meal composition. Recently we examined the response of muscle protein metabolism to ingestion of a bolus solution of 6 g of essential amino acids and 35 g of carbohydrates after an intense resistance exercise bout (86). After the amino acid ingestion, arterial amino acid levels increased ~3-fold, and insulin increased ~10-fold. Muscle protein synthesis was increased 3.5-fold, and the expected rise in muscle protein breakdown was inhibited. Thus, a very small amount of amino acids plus a substantial amount of carbohydrates consumed postexercise changed net muscle protein balance from negative to positive, that is, from a catabolic state to an anabolic state. Also, infusion of amino acids plus glucose resulted in a switch from catabolism to anabolism in the muscles of dogs after treadmill exercise (78).

## SUBCELLULAR MECHANISMS INVOLVED IN THE RESPONSE OF MUSCLE PROTEIN SYNTHESIS TO CONTRACTILE ACTIVITY

### Effects of Exercise Alone

Changes in the accustomed level of physical activity will alter the composition of muscle as a result of the differential expression of various genes (17, 47). However, it is not known how such transcriptional changes are regulated by contractile events, and, in any case, a major portion of the responses to exercise occur too rapidly for gene transcription to be involved (e.g. acute alteration of protein turnover).

Increases in rates of muscle protein synthesis are most likely the result of increased translational efficiency. The molecular mechanisms involved in the responses to exercise have just begun to be examined. The proteins involved are eukaryotic initiation factors (eIF), particularly eIF4E, which, when it forms a complex with eIF4G, promotes the initiation of protein synthesis. This is normally prevented when the available eIF4E is bound to a phosphorylatable binding protein, eIF4-BP1, phosphorylation of which releases eIF4E. In rats, when muscle protein synthesis decreased 26% as a result of treadmill running, there was a four-fold increase in the amount of eIF4E in the inactive form (43). Additionally, eIF4E association with eIF4G was decreased 71%. When rats were fed after exercise, muscle protein synthesis was restored to control rates, and this was associated with an increase in the active form of eIF4E and the eIF4E-eIF4G complex. The amount of inactive eIF4E was inversely correlated to the rate of muscle protein synthesis, and the amounts of phosphorylated eIF4BP-1 and of eIF4E-eIF4G complex were positively correlated. After resistance exercise in rats, the increase in muscle

protein synthesis was related to an increase in another protein, eIF2B (35), which is involved in the stimulation of general protein synthesis. In a study investigating the effects of contraction on muscle protein synthesis in human quadriceps, 10 sets of eight repetitions at 80% of 1RM elicited a rise in the amount of eIF4-BP1, which correlates with the amount of eIF4F in the free active form (92).

An important component of the hormone/nutrient sensing and signaling pathways that influences translation is P70S6 kinase, which stimulates initiation of protein synthesis after phosphorylation of the ribosomal S6 protein and which itself is the target of the mTOR (mammalian target of rapamycin) protein in the PI3 kinase signaling pathway. The involvement of this protein in the contraction-stimulated response of protein synthesis was neatly described by a recent study, which demonstrated a marked increase in its activity after lengthening contractions of the soleus and anterior tibialis (4). Polyribosome profiles demonstrated that the increase in protein synthesis was caused by increased translation; the increases in P70S6K activity changes 6 h after the contractile stimulus correlated well with the eventual extent of hypertrophy.

## Effects of Exercise and Feeding

Rats refed leucine alone after severe treadmill exercise had muscle protein synthesis that was greater than in fasted rats (2), demonstrating that a single amino acid may stimulate the response of muscle protein synthesis. Leucine given in a bolus dose can also stimulate protein synthesis (100) in human subjects at rest.

When the rats that had been subjected to a bout of treadmill exercise were refed a mixed carbohydrate and amino acid meal, the expected increase in muscle protein synthesis was associated with a decrease in inactive eIF4E and an increase in eIF4E binding to eIF4G (43). However, when rats were fed a solely carbohydrate meal, isoenergetic with the mixed meal, there was neither an increase in muscle protein synthesis nor any significant change in inactive eIF4E or eIF4G binding with eIF4G. Insulin availability was similar during the two meals. These data suggest that amino acids from the mixed meal played a role in signaling translation initiation for muscle protein synthesis, as they do at rest.

The stimulatory effect of amino acids appears to be enhanced by an enhancement of eIF4-BP1 phosphorylation after exercise in human subjects (92). The effect of contractile activity stimulation was additive to that of amino acids and, because the amino acid effect alone appeared to be maximal, the pathway by which contraction is signaled may be distinct.

## PROTEIN DIETARY REQUIREMENTS FOR PHYSICALLY ACTIVE PEOPLE

The protein dietary requirements for physically active people is a topic that has been reviewed on a number of occasions (21, 87, 88). The important question is, does habitual physical activity involving dynamic exercise such as running, in which

amino acids are oxidized, or resistance exercise, causing muscle hypertrophy, increase dietary protein requirements? For reasons explained in detail by Millward (72), it is very difficult to absolutely settle these questions, because of a number of technical problems (e.g. the difficulty of studying subjects who were in a steady state after a change from one test diet to another). None of the studies that led their authors to conclude that there were additional protein requirements have overcome these problems.

## Dynamic Exercise

Exercise increases amino acid oxidation, particularly of the BCAAs, and most workers in the field are willing to accept that amino acid oxidation leads to increased ureagenesis and loss of nitrogen. People who have high habitual energy expenditure must maintain energy balance or lose body mass. When intense physical activity is associated with insufficient input (e.g. owing to anorexia during mountaineering at high altitude), wasting of lean-tissue mass is inevitable unless an eating protocol is established (22). The controversial question concerns the possibility that accustomed exercise will place, on physically active people, an additional burden of eating more protein than they would otherwise do. Lemon (65), Phillips et al (79), and Tarnopolsky et al (103) have concluded that this is the case, but in our view the evidence is insufficient as well as biologically counter-intuitive.

As Butterfield has pointed out (21), most of the studies suggesting enhanced protein requirements for physically active individuals were not done in conditions in which training state, exercise intensity, or energy balance were sufficiently steady to make robust observations of protein and amino acid metabolism. The classic study by Butterfield & Calloway (23) demonstrated that an increased level of physical activity actually increased the efficiency of protein utilization. Furthermore, most of the extra exercise-induced amino acid oxidation is BCAA oxidation, and this is not greater in well-trained individuals (62) exercising at the same relative workload. It has been argued that the volume of training undertaken by some athletes is sufficient to make it difficult for them to meet protein requirements. However, the evidence that elite touring cyclists (such as those competing in the Tour de France) manage to maintain lean body mass adequately despite energy expenditures of 25–35 MJ/day (96) argues against this. Although touring cyclists have some difficulty covering their energy requirements, when they do, they achieve a nitrogen balance insignificantly different from zero (20).

## Resistance Exercise

Weight training causes little or no effect on whole-body leucine oxidation (104), suggesting that the use of protein as a fuel is not a consideration in this kind of activity. Thus, the question becomes whether resistance exercise causes an increased need for protein to allow remodeling of muscle. Because this kind of exercise is anabolic acutely, but training results in adaptation (see above), the conclusion is the same as that reached by Butterfield & Calloway; that is, exercise

makes the use of protein more efficient, thus decreasing protein requirements, not increasing them! In fact, eating more protein habitually leads to a rapid loss of body protein if the accustomed diet is suddenly diminished (88), so mountaineers and explorers should ideally accustom themselves to a low-protein diet before venturing onto the heights or into the wilderness.

## Recommendations for Requirements

As long as energy balance is achieved and food of a normal protein content (12%–15% of total energy) is consumed, even athletes in training should not require any further protein supplementation of their diet. As far as we can see from the literature, there are no factors, such as age or gender, that modify this.

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